

02/25/99

Practitioner's Docket No. U 012121-2

PATENT

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Box SEA

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application  
 Assistant Commissioner for Patents  
 Washington, D.C. 20231



## NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of  
 Inventor(s):

1. MAARTEN HENDRIK STUIVER
2. JERÔME HUBERTINA HENRICUS VICTOR CUSTERS
3. MARIANNE BEATRIX SELA-BUURLAGE
4. LEO SJOERD MELCHERS
5. WESSEL LAGEWEG
6. ANNE SILENE PONSTEIN
7. JOHANNA PIETERNELLA ELS VAN DEVENTER-TROSST

**WARNING:** 37 CFR 1.41(a)(1) points out:

*"(a) A patent is applied for in the name or names of the actual inventor or inventors*

*(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."*

For (title):

ANTIFUNGAL PROTEINS, DNA CODING THEREFORE, AND HOSTS  
 INCORPORATING SAME

## CERTIFICATION UNDER 37 C.F.R. 1.10\*

(Express Mail label number is **mandatory**.)

(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date FEBRUARY 25, 1999, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EJ405329117US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JENIFFER RASHKIN

(type or print name of person mailing paper)

*Jennifer Rashkin*  
 Signature of person mailing paper

**WARNING:** Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

**\*WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).

*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct 24, 1996, 60 Fed. Reg. 56,439, at 56,442*

## 1. Type of Application

This new application is for a(n)

(check one applicable item below)

- ☐ Original (nonprovisional)
- ☐ Design
- ☐ Plant

**WARNING:** Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.

**WARNING:** Do not use this transmittal for the filing of a provisional application.

**NOTE:** If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION

- ☐ Divisional.
- ☒ Continuation.
- ☐ Continuation-in-part (C-I-P).

## 2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

**NOTE:** A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America, or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 CFR 1.78(a)(1).

**NOTE** If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

**WARNING:** If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b) ) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not,

the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

**WARNING:** When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).

- ☒ The new application being transmitted claims the benefit of prior U.S. application(s).  
Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL  
WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

### 3. Papers Enclosed

#### A. Required for Filing Date under 37 C.F.R. 1.53(b) (Regular) or 37 C.F.R. 1.153 (Design) Application

116 Pages of Specification  
5 Pages of Claims  
12 Sheets of Drawing  
☒ Formal  
☐ Informal

#### B. Other Papers Enclosed

1 Pages of Abstract  
Other

**WARNING:** **DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988 . . . (1990 O.G. 57-62).

**NOTE:** "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page." 37 C.F.R. 1.84(c).

(complete the following, if applicable)

- ☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

### 4. Additional Papers Enclosed

- ☐ Preliminary Amendment  
☒ Information Disclosure Statement (37 C.F.R. 1.98)  
☒ Form PTO-1449 (PTO/SB/08A and 08B)  
☒ Citations  
☒ Declaration of Biological Deposit  
☒ Submission of "Sequence Listing," computer readable copy and/or amendment

pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

- ☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative  
☐ Special Comments  
☐ Other

## 5. Declaration or Oath

**NOTE:** *A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 CFR 1.63(d).*

**NOTE:** *A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor and state whether the inventor is a sole or joint inventor. 37 CFR 1.63(a)(1)-(4)*

- ☐ Enclosed  
Executed by

*(check all applicable boxes)*

- ☐ inventor(s).  
☐ legal representative of inventor(s). 37 CFR 1.42 or 1.43.  
☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.  
☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

- ☒ Not Enclosed.

**NOTE:** *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

- ☒ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

*(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e),  
can be filed subsequently).*

**NOTE:** *It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b)*

- ☐ Showing that the filing is authorized.  
*(not required unless called into question. 37 CFR 1.41(d))*



## 6. Inventorship Statement

**WARNING:** *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted*

The inventorship for all the claims in this application are:

☐ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

## 7. Language

**NOTE:** *An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of \$130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).*

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

## 8. Assignment

☒ An assignment of the invention to MOGEN INTERNATIONAL N.V.

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☒ will follow.

**NOTE:** *"If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).*

**WARNING:** *A newly executed "STATEMENT UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O G 62-64*

## 9. Certified Copy

Certified copy(ies) of application(s)

Country

Appln. no.

Filed

Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

#### 10. Fee Calculation (37 C.F.R. 1.16)

A. ☒ Regular application

CLAIMS AS FILED					
Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. 1.16(a) \$760.00
Total Claims (37 CFR 1.16(c))	50	- 20 = 0	30 x	\$ 18.00	\$ 540.00
Independent Claims (37 CFR 1.16(b))	12	- 3 = 0	9	\$ 78.00	\$ 702.00
Multiple Dependent Claim(s), if any (37 CFR 1.16(d))			+	\$260.00	

☐ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☒ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation \$ 2,002.00

- B. ☐ Design application  
(\$310.00—37 CFR 1.16(f))

Filing Fee Calculation \$ \_\_\_\_\_

- C. ☐ Plant application  
(\$480.00—37 CFR 1.16(g))

Filing Fee Calculation \$ \_\_\_\_\_

11. Small Entity Statement(s)

- ☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

**WARNING:**

*"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section " 37 CFR 1.28(a)(2)*

*(complete the following, if applicable)*

- ☐ Status as a small entity was claimed in prior application  
\_\_\_\_\_/\_\_\_\_\_, filed on \_\_\_\_\_ from which benefit is being claimed  
for this application under:

35 U.S.C. § ☐ 119(e),  
☐ 120,  
☐ 121,  
☐ 365(c),

and which status as a small entity is still proper and desired.

- ☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of A, B or C above) \$ \_\_\_\_\_

**NOTE:** Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee The two-month period is not extendable under § 1.136. 37 CFR 1.28(a).

**12. Request for International-Type Search (37 C.F.R. 1.104(d))**

*(complete, if applicable)*

- ☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

**13. Fee Payment Being Made at This Time**

- ☒ Not Enclosed

☒ No filing fee is to be paid at this time.  
*(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)*

- ☐ Enclosed

☐ Filing fee \$ \_\_\_\_\_

☐ Recording assignment  
(\$40.00; 37 C.F.R. 1.21(h))  
(See attached "COVER SHEET FOR  
ASSIGNMENT ACCOMPANYING NEW  
APPLICATION.") \$ \_\_\_\_\_

☐ Petition fee for filing by other  
than all the inventors or person  
on behalf of the inventor where  
inventor refused to sign or cannot  
be reached  
(\$130.00; 37 C.F.R. 1.47 and 1.17(i)) \$ \_\_\_\_\_

☐ For processing an application with a  
specification in a non-English language  
(\$130.00; 37 C.F.R. 1.52(d) and 1.17(k)) \$ \_\_\_\_\_

☐ Processing and retention fee  
(\$130.00; 37 C.F.R. 1.53(d) and 1.21(l)) \$ \_\_\_\_\_

☐ Fee for international-type search report  
(\$40.00; 37 C.F.R. 1.21(e)) \$ \_\_\_\_\_

**NOTE:** 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f)

Total Fees Enclosed \$ \_\_\_\_\_

**14. Method of Payment of Fees**

- ☐ Check in the amount of \$\_\_\_\_\_
- ☐ Charge Account No. \_\_\_\_\_ in the amount of \$\_\_\_\_\_.  
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid 37 CFR 1.22(b).

**15. Authorization to Charge Additional Fees**

**WARNING:** If no fees are to be paid on filing, the following items should not be completed.

**WARNING:** Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

- ☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No.\_\_\_\_\_.
- ☐ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)
- ☐ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

- ☐ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)
- ☐ 37 CFR 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).
- ☐ 37 C.F.R. 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 CFR 1.136(a)(3).

- ☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b)).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

**16. Instructions as to Overpayment**

NOTE: "... Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 CFR 1.26(a).

☐ Credit Account No. \_\_\_\_\_

☐ Refund

Reg. No. 30,086

Tel. No.: (212)708-1890

Customer No.:

  
**SIGNATURE OF PRACTITIONER**

**CLIFFORD J. MASS**

(type or print name of practitioner)

**LADAS & PARRY**

P.O. Address

**26 WEST 61ST STREET**

**NEW YORK, NEW YORK 10023**

☐ **Incorporation by reference of added pages**

*(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)*

☒ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added 5

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added \_\_\_\_\_

☐ Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added \_\_\_\_\_

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added \_\_\_\_\_

☐ **Statement Where No Further Pages Added**

*(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)*

☐ This transmittal ends with this page.

**ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF  
PRIOR U.S. APPLICATION(S) CLAIMED**

NOTE: See 37 CFR 1.78.

**17. Relate Back**

**WARNING:** If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

(complete the following, if applicable)

☐ Amend the specification by inserting, before the first line, the following sentence:

**A. 35 U.S.C. 119(e)**

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4)

☐ "This application claims the benefit of U.S. Provisional Application(s) No(s).:

**APPLICATION NO(S):**

**FILING DATE**

\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_

\_\_\_\_\_"  
\_\_\_\_\_"  
\_\_\_\_\_"

**B. 35 U.S.C. 120, 121 and 365(c)**

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

☒ "This application is a

☒ continuation

☐ continuation-in-part

☐ divisional

of copending application(s)

☐ application numbers \_\_\_\_\_ filed on \_\_\_\_\_

☒ International Application PCT/EP97/04923 filed on 4 SEPTEMBER 1997 and which designated the U.S., claims the benefit thereof and incorporates the same by reference."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

☐ "The nonprovisional application designated above, namely application \_\_\_\_\_ / \_\_\_\_\_, filed \_\_\_\_\_ claims the benefit of U.S. Provisional Application(s) No(s).:



APPLICATION NO(S).:

FILING DATE

\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_

\_\_\_\_\_"  
\_\_\_\_\_"  
\_\_\_\_\_"

☐ Where more than one reference is made above please combine all references into one sentence.

**18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application**

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

EP 96202466.7 SEPTEMBER 4, 1996

EP 97200831.2 MARCH 19, 1997

Country Appln. no. Filed

The certified copy(ies) has (have)

☒ been filed in prior application PCT/EP97/04923, which was filed on 4 SEPTEMBER 1997.

☐ is (are) attached.

**WARNING:** The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).

**19. Maintenance of Copendency of Prior Application**

**NOTE:** The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O G 27).

A. ☐ Extension of time in prior application

(This item **must** be completed and the papers filed in the **prior application**, if the period set in the prior application has run.)

☐ A petition, fee and response extends the term in the pending **prior** application until\_\_\_\_\_.

☐ A **copy** of the petition filed in prior application is attached.

B. ☐ Conditional Petition for Extension of Time in Prior Application

*(complete this item, if previous item not applicable)*

☐ A conditional petition for extension of time is being filed in the pending **prior** application.

☐ A **copy** of the conditional petition filed in the prior application is attached.

**20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed**

*(complete applicable item (a), (b) and/or (c) below)*

(a) ☐ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

☐ the same.

☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

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*(type name(s) of inventor(s) to be deleted)*

(b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

☐ the same.

☐ the following additional inventor(s) have been added:

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*(type name(s) of inventor(s) to be deleted)*

(c) ☐ The inventorship for all the claims in this application are

☐ the same.

☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

☐ is submitted.

☐ will be submitted.

**21. Abandonment of Prior Application *(if applicable)***

☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application

is granted a filing date, so as to make this application copending with said prior application.

*NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.*

## 22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

**WARNING:** "The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b).

*NOTE: Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.*

*(check the next item, if applicable)*

- ☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

## 23. Small Entity (37 CFR § 1.28(a))

- ☐ Applicant has established small entity status by the filing of a statement in parent application \_\_\_\_\_ on \_\_\_\_\_.

- ☐ A copy of the statement previously filed is included.

**WARNING:** See 37 CFR § 1.28(a).

## 24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

- ☐ A notification of the filing of this  
*(check one of the following)*

- ☐ continuation

- ☐ continuation-in-part

- ☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

ANTIFUNGAL PROTEINS, DNA CODING THEREFORE, AND HOSTS  
INCORPORATING SAME

FIELD OF THE INVENTION

5           The present invention relates to new oxidases, which can act as antifungal proteins, DNA coding therefor and hosts incorporating the DNA, as well as methods of combating fungal pathogens by causing said fungal pathogens to be contacted with said protein or proteins. The invention further relates to plants, incorporating and expressing  
10 DNA coding for antifungal proteins, and to plants which as a result thereof show reduced susceptibility to fungal pathogens.

BACKGROUND ART

          Fungal diseases of crop plants have been one of the principal  
15 causes of crop losses throughout the history of crop cultivation. The growing of crops as monocultures encourages the proliferation of virulent races of fungal pathogens and wherever a new variety of crop plant becomes grown on a wide scale of the risks of a virulent strain of a pathogen evolving to attack that crop increase drastically. The  
20 occurrence of disease is significantly worsened by the international transport of pathogen-carrying plant materials, which can bring together plants with pathogens against which they have had no opportunity to evolve resistance. Thus by man's intervention the natural balance between host and pathogen has been disturbed with  
25 disastrous effect on a number of occasions. Catastrophic losses and even famines such as occurred in Ireland during the 19th century, caused by the potato blight fungus (*Phytophthora infestans*) have resulted from such activities. Fungal disease can also make it completely impossible to grow certain crops in large areas, as was the  
30 case when *Fusarium* wilt wiped out tomato growing in large areas of the Eastern USA or the downy mildew (*Plasmopara viticola*) fungus devastated vine growing in parts of Europe. Outbreaks of fungal disease can also have a severe effect on the environment as happened when almost the entire English Elm (*Ulmus procera*) population was  
35 destroyed by Dutch Elm Disease (*Ceratocystis ulmi*). In addition the losses which may be caused during the growing of crops fungal disease may contribute to further post harvest losses. Various soft rots such as *Botrytis cinerea* are particularly problematic in soft fruit, for

example. The fungus *Aspergillus flavus*, although not a true disease-causing fungus, causes post-harvest rot on stored peanuts and maize, especially in tropical countries and is most serious because it produces a toxin, aflatoxin, which is very toxic to man.

5

The major economic problems associated with fungal diseases are found in wetter parts of the World, principally Western Europe and the humid tropics. Various crop husbandry techniques, such as crop rotation and avoiding the spread of soil on machinery etc., are used to prevent the build up and spread of severe infestations of fungal disease. Plant breeding has made a significant impact on improving the resistance of many crops to important diseases. For example, plant breeders successfully introduced resistance genes 1 and 1-2 effective against *Fusarium oxysporum* f.sp. *lycopersici* into tomato. Nonetheless, problems remain, particularly when many forms of race-specific resistance break down as new races of the pathogen rapidly evolve. In tomato, another virulent strain of *F. oxysporum* has occurred and breeders are seeking a third useful resistance gene. In cereals growing in parts of Western Europe a recent outbreak of a virulent strain of yellow rust (*Puccinia striiformis*) has lead to a rapid increase in fungicide use on varieties which remain resistant to other fungi. In these specific cases chemicals are widely used to control fungal disease, as in cases where there are simply no natural sources of resistance available to the breeder.

Chemical fungicides remain a major input in the costs of crop production in many parts of the World. In 1990 21% of all agrochemical sales were accounted for by fungicides (US \$ 5,54 million). Farmers and growers have a strong motivation to reduce their input costs. Added to the economic justification is an increasingly strong environmental component in the equation. There is growing pressure in the more advanced economies, notably in North America and Western Europe, from politicians and consumers for agriculture which relies less on chemical inputs. The justification for such demands may lack focused rationale or scientific proof, but fears grow with reports of pesticides traced in groundwater or detected in quantities exceeding the minimums acceptable as food residues. In The Netherlands, for example, there is a mandatory requirement to reduce total pesticides use by 50% before 2000.

*Phytophthora infestans* belongs to the group of fungi referred to as Oomycetes. *Phytophthora infestans* infects various members of Solanaceae, such as potato, tomato and some ornamentals. It causes late blight of potatoes and tomatoes affecting all parts except roots. Geographically, the fungus is widely distributed, and it can be found in all potato-producing countries. Economically late blight in potatoes is of major importance, as infection early in the season can severely reduce crop yield. Currently the disease is controlled by spraying chemical fungicides (dithiocarbamates, such as mancozeb, manec and zineb) regularly. Both from an environmental and economical point of view, biological control of diseases caused by *Phytophthora infestans* could have advantages over the use of chemical fungicides.

*Pythium* also belongs to the group of fungi referred to as Oomycetes. The genus *Pythium* differs from the related genus *Phytophthora* by forming relatively undifferentiated sporangia. Geographically, this fungus is widely distributed on all continents. The first main type of disease caused by *Pythium* species is damping-off, due to sudden and fast developing attacks on young seedlings in the field or in nurseries. *Pythium* species cause a second type of disease which is root necrosis and causes a general slowing of plant growth (for example wheat and maize) and loss of yield. The main losses caused by *Pythium* in Europe are to field crops such as sugarbeet. In principle, losses tend to be all-or-nothing. Similarly, nursery sowings of ornamentals and forest trees may be completely destroyed. (For a review on Oomycetes, vide: European Handbook of Plant Diseases, ed. by I.M. Smith et al., 1988, Blackwell Scientific Publications, Ch.8)

Another fungus is *Botrytis*, especially *B. cinerea*, belonging to the group of *Fungi Imperfecti*, which causes gray mold blight or bud and flower blight, which is common on soft ripe fruits after harvesting, but it can also occur before harvest. It can also affect various vegetables such as lettuce, beans and tomato. Other species of *Botrytis* are common on flowers, such as lilies, gladiolus and tulips.

A protein with antifungal activity, isolated from TMV-induced tobacco leaves, which is capable of causing lysis of germinating spores and hyphal tips of *Phytophthora infestans* and which causes the hyphae to grow at a reduced rate, was disclosed in WO91/18984 A1. This protein has an apparent molecular weight of about 24 kDa and was named

AP24. Comparison of its complete amino acid sequence, as deduced from the nucleic acid sequence of the AP24 gene, with proteins known from databases revealed that the protein was an osmotin-like protein.

Despite initial success in combating fungal pathogens, such as *Phytophthora infestans*, and the genetic engineering of plants capable of producing these antifungal proteins with activity against this fungal pathogen there remains a need to identify and isolate other proteins with antifungal activity against this fungus.

#### SUMMARY OF THE INVENTION

The present invention provides an isolated protein obtainable from a plant source which has antifungal activity, most effectively directed to Oomycetes, and preferably to *Phytophthora* and/or *Pythium* and a molecular weight of about 55-65 kDa as judged by SDS PAGE-electrophoresis. Preferred proteins are those that are obtainable from sunflower or lettuce plants. Even more preferred proteins are obtainable from sunflower or lettuce leaves induced with sodium salicylate. A still more preferred isolated protein is characterised in that it is selected from the group of proteins having the amino acid sequence selected from the group comprising of the amino acid sequences depicted in SEQ ID NO's: 1, 2 or 6, 16, 20, 49, 50, 51, 58, 71, 73 or 75 as well as muteins thereof which have antifungal and especially anti-*Phytophthora* and/or anti-*Pythium* activity. A still further preferred protein according to the invention is one characterised in that it comprises a protein that comprises the amino acid sequence as represented by SEQ ID NO's: 16, 20, 58, 71, 73 or 75 or by a part of said sequence like represented in SEQ ID NO: 6.

The invention also provides a new enzyme, the enzymatic activity being oxidation of carbohydrates.

The invention also embraces an isolated DNA sequence comprising an open reading frame capable of encoding a protein according to the invention, preferably characterised in that the open reading frame is capable of encoding a protein according to the invention, and DNA capable of hybridising therewith under stringent conditions.

The invention also provides a chimeric DNA sequence according to the invention further comprising a transcriptional initiation region and, optionally, a transcriptional termination region, so linked to said open reading frame as to enable the DNA to be transcribed in a

living host cell when present therein, thereby producing RNA which comprises said open reading frame. A preferred chimeric DNA sequence according to the invention is one, wherein the RNA comprising said open reading frame is capable of being translated into protein in said host cell, when present therein, thereby producing said protein. Especially preferred are DNA sequences comprising a sequence as depicted in SEQ ID NO's: 15, 19, 57, 70, 72 or 74.

The invention also embraces a chimeric DNA sequence comprising a DNA sequence according to the invention, which may be selected from replicons, such as bacterial cloning plasmids and vectors, such as a bacterial expression vector, a (non-integrative) plant viral vector, a Ti-plasmid vector of *Agrobacterium*, such as a binary vector, and the like, as well as a host cell comprising a replicon or vector according to the invention, and which is capable of maintaining said replicon once present therein. Preferred according to that embodiment is a host cell which is a plant cell, said vector being a non-integrative viral vector.

The invention further provides a host cell stably incorporating in its genome a chimeric DNA sequence according to the invention, such as a plant cell, as well as multicellular hosts comprising such cells, or essentially consisting of such cells, such as plants. Especially preferred are plants characterised in that the chimeric DNA according to the invention is expressed in at least a number of the plant's cells causing the said antifungal protein to be produced therein.

According to yet another embodiment of the invention a method for producing a protein with carbohydrate oxidase activity is provided, characterised in that a host cell according to the invention is grown under conditions allowing the said protein to be produced by said host cell, optionally followed by the step of recovering the protein from the host cells.

Another part of the invention is directed to the antifungal use of a protein which has carbohydrate oxidase activity.

The invention provides also for the use of a protein according to the invention for retarding the growth of fungi, preferably Oomycetes and more preferably *Phytophthora* and *Pythium*. According to yet another embodiment, retarding the growth of the fungi is on or in the neighbourhood of the plant by applying a microorganism capable of producing the protein or by harvesting the protein from a microbial



host and applying the protein in an agrochemical formulation.

The invention also provides a method for obtaining plants with reduced susceptibility to fungi, especially *Phytophthora* and/or *Pythium*, comprising the steps of

- 5 (a) introducing into ancestor cells which are susceptible of regeneration into a whole plant,
  - a chimeric DNA sequence comprising an open reading frame capable of encoding a protein according to claim 1, said open reading frame being operatively linked to a transcriptional and translational region
  - 10 and, optionally, a transcriptional termination region, allowing the said protein to be produced in a plant cell that is susceptible to infection by said fungus, and
  - a chimeric DNA sequence capable of encoding a plant selectable marker allowing selection of transformed ancestor cells when said
  - 15 selectable marker is present therein, and
- (b) regenerating said ancestor cells into plants under conditions favouring ancestor cells which have the said selectable marker, and
- (c) identifying a plant which produces a protein according to claim 1, thereby reducing the susceptibility of said plant to infection by said
- 20 fungus.

Preferred according to the invention is a method characterised in that step (a) is performed using an *Agrobacterium tumefaciens* strain capable of T-DNA transfer to plant cells and which harbours the said chimeric DNA cloned into binary vector pMOG800; another preferred

25 method is when step (b) is performed in the presence of an antibiotic favouring cells which have a neomycin phosphotransferase.

The invention further provides an antifungal composition comprising a protein according to the invention and a suitable carrier.

- 30 An antibody, capable of reacting with an N-terminal fragment of a protein according to the invention, preferably to the peptide represented by SEQ ID NO's: 6, 16, 20, 58, 71, 73 or 75 is also provided. The antibody is suitably used to detect expression levels of chimeric DNA according to the invention in host cells and
- 35 multicellular hosts, preferably plants, capable of producing a protein according to the invention.

The invention also provides a nucleic acid sequence obtainable from a gene encoding a protein according to the invention, said nucleic acid

sequence having tissue-specific transcriptional regulatory activity in a plant. The invention specifically provides a nucleic acid sequence obtainable from the region upstream of the translational initiation site of said gene, preferably at least 500 nucleotides immediately upstream of the translational initiation site of said gene.

#### DESCRIPTION OF THE FIGURES

Figure 1: SDS-PAGE (12.5%) of the different purification steps of MS59 sunflower protein. Mw= molecular weight markers; 1= crude sunflower protein extract after gel filtration (G25); 2= protein fraction bound to cation exchange chromatography (S-sepharose); 3= pool of active fractions after cation exchange chromatography (Mono S); 4= flow through from hydrophobic interaction chromatography (phenyl superose); 5= active fractions after gel filtration.

Figure 2: SDS-PAGE (12.5%) of different fractions (number 6 to 16) of the gelfiltration (SD75) column. Fraction 10 to 15 was tested in 3 dilutions for growth inhibition on *Phytophthora infestans* (PANEL A) and on *Pythium ultimum* (PANEL B)

Figure 3: SDS-PAGE (12.5%) of fractions eluted from nine gel slices (lane 1 to 9) of a native PAGE in which a MS59 containing SD75 fraction (SD75 fraction 13) was separated. Right panel: SDS-PAGE (12.5%) with SD75 fraction 13 (L) and two fractions of elution experiment fraction 2 (with MS59) and fraction 5 (with a ~30 kD protein). Bottom panel: growth inhibition of *Phytophthora infestans* tested with elution fraction 1 to 6, with 5 µl and 1 µl added per well.

Figure 4: Microscopical analysis of an in vitro fungal inhibition assay 24 hours after addition of *Phytophthora infestans* zoospores to PDA medium. Left panel: control incubation, only MES buffer was added. Right panel: *E. coli*-produced MS59 in MES buffer was added to the incubation.

Figure 5: Microscopical analysis of an *in vitro* fungal inhibition assay 24 hours after addition of *Pythium ultimum* hyphal fragments to PDA medium. Left panel: control incubation, only MES buffer was added. Right panel: *E. coli*-produced MS59 in MES buffer was added to the incubation.

Figure 6: (A). SD 75 gelfiltration profile of WL64. WL64 eluates at fractions 13, 14, 15. Molecular weight markers are indicated above the arrows at the top of the plot. X-axis: fraction number. Y-axis: A280.

(B). Coomassie stained 12.5% SDS-PAGE gel of fractions 11-17 of the SD 75 gelfiltration profile. Molecular weight markers are indicated on the right and are in kDa. The protein bands that correlate with antifungal activity are indicated between the arrows.

(C). *In vitro* antifungal assay. Ten microlitres of the respective fractions (500  $\mu$ l total) were used to screen the growth inhibition of *Rhizoctonia solani* hyphal fragments.

Figure 7: Coomassie stained 12.5% SDS-PAGE gel of the purification of WL64. Lane 1, lettuce extract; lane 2, HIC peak; lane 3, Source S peak; lane 4, Mono S peak; lane 5, SD 75 peak; lane 6, Mono P peak. Molecular weight markers are indicated on both sides of the figure and are in kDa.

Figure 8: (A). Lineweaver-Burk plot of MS59 (open diamonds), WL64 (closed circles), and GOX (open squares) oxidase activities with glucose as substrate. Amounts of protein per assay were 17, 29, and 45 ng for MS59, WL64 and GOX respectively.

(B). Lineweaver-Burk plot of MS59 (open diamonds), WL64 (closed circles), and GOX (open squares) oxidase activities with fungal cell walls as substrate. Amounts of protein per assay were 17, 29, and 225 ng for MS59, WL64 and GOX respectively.

Figure 9: Substrate specificity for the oxidase activities of MS59 (dotted bars), WL64 (diagonal striped bars), and GOX (filled bars).

Figure 10: Alignment of the proteins of the invention MS59, WL64 and the two homologues from *A. thaliana* At26 (SEQ ID NO: 71) and At27 (SEQ ID NO: 75) (with the known berberine bridge enzymes (EcBBE and PsBBE). Conserved changes are denoted in gray, while areas of identity (3 of the 6 amino acids identical) are given in black.

#### DETAILED DESCRIPTION OF THE INVENTION

The antifungal effect of the protein(s) of the invention has been demonstrated in *in vitro* assays for the following fungi;

10 *Phytophthora infestans*, *Phytophthora cactorum*, *Phytophthora nicotiana*, *Phytophthora megasperma*, *Pythium ultimum*, *Pythium sylvaticum*, *Pythium violae*, *Pythium paroecandrum*, *Rhizoctonia solani*, *Tanatephorus cucumeris*, *Helicobasidium purpureum*, *Sclerotium cepivorum*, *Pichia pastoris* and *Botrytis cinerea* for purposes of illustration. It will be

15 clear, that the use of the protein(s) of the invention, or DNA encoding therefore, for use in a process of combating fungi is not limited to the mentioned fungi. There is no reason to assume that the protein(s) according to the invention do not possess antifungal activity against a far broader range of fungi than those tested here,

20 especially in the class of Oomycetes.

Although the invention is illustrated in detail for transgenic tomato, tobacco, carrot, potato and *Brassica napus* plants, it should be understood that any plant species that is subject to some form of

25 fungal attack, especially from the fungi mentioned above, may be provided with one or more plant expressible gene constructs, which when expressed overproduce the protein(s) of the invention in said plant in order to decrease the rate of infectivity and/or the effects of such attack. The invention can even be practiced in plant species

30 that are presently not amenable for transformation, as the amenability of such species is just a matter of time and because transformation as such is of no relevance for the principles underlying the invention. Hence, plants for the purpose of this description shall include angiosperms as well as gymnosperms, monocotyledonous as well as

35 dicotyledonous plants, be they for feed, food or industrial processing purposes; included are plants used for any agricultural or horticultural purpose including forestry and flower culture, as well as home gardening or indoor gardening, or other decorative purposes.

The protein according to the present invention may be obtained by isolating it from any suitable plant source material containing it. A particularly suitable source comprises leaves of the sunflower (*Helianthus*) and leaves of lettuce (*Lactuca sativa* cv. Lollo bionda).

- 5 The presence of antifungal proteins according to the invention in plant source material can readily be determined for any plant species by making plant extracts from those species and testing those extracts for the presence of antifungal activity using *in vitro* antifungal assays as described herein, further fractionating the obtained samples
- 10 by any suitable protein fractionation technique in conjunction with the *in vitro* assay until an antifungal fraction is obtained which comprises an approximately 55-65 kDa protein, internally denoted as MS59 or its homologue WL64, which in isolated form shows antifungal activity. Especially, fractions may be tested for antifungal activity
- 15 on Oomycetes, for example, *Phytophthora* or *Pythium ultimum* and the like, or other fungi, such as the Basidiomycetes, Ascomycetes, Zygomycetes or other classes or subclasses.

- Alternatively, antifungal proteins according to the invention may be obtained by cloning DNA comprising an open reading frame
- 20 capable of encoding said protein, or the precursor thereof, linking said open reading frame to a transcriptional, and optionally a translational initiation and transcriptional termination region, inserting said DNA into a suitable host cell and allowing said host cell to produce said protein. Subsequently, the protein may be
- 25 recovered from said host cells, preferably after secretion of the protein into the culture medium by said host cells. Alternatively, said host cells may be used directly in a process of combating fungal pathogens according to the invention as a pesticidal acceptable composition.

- 30 Host cells suitable for use in a process of obtaining a protein according to the invention may be selected from prokaryotic microbial hosts, such as bacteria e.g. *Agrobacterium*, *Bacillus*, *Cyanobacteria*, *E.coli*, *Pseudomonas*, and the like, as well as eukaryotic hosts including yeasts, e.g. *Saccharomyces cerevisiae*, fungi, e.g.
- 35 *Trichoderma* and plant cells, including protoplasts.

In a method of retarding the growth of the fungi on or in the neighbourhood of the plant leaves, host cells may suitably be selected from any species routinely used as biological fungicides.

Also the proteins can be produced by microorganisms, harvested and applied in a agrochemical formulation.

The word protein means a sequence of amino acids connected through peptide bonds. Polypeptides or peptides are also considered to be proteins. Muteins of the protein of the invention are proteins that are obtained from the proteins depicted in the sequence listing by replacing, adding and/or deleting one or more amino acids, while still retaining their antifungal activity. Such muteins can readily be made by protein engineering *in vivo*, e.g. by changing the open reading frame capable of encoding the antifungal protein such that the amino acid sequence is thereby affected. As long as the changes in the amino acid sequences do not altogether abolish the antifungal activity such muteins are embraced in the present invention.

The present invention provides a chimeric DNA sequence which comprises an open reading frame capable of encoding a protein according to the invention. The expression chimeric DNA sequence shall mean to comprise any DNA sequence which comprises DNA sequences not naturally found in nature. For instance, chimeric DNA shall mean to comprise DNA comprising the said open reading frame in a non-natural location of the plant genome, notwithstanding the fact that said plant genome normally contains a copy of the said open reading frame in its natural chromosomal location. Similarly, the said open reading frame may be incorporated in the plant genome wherein it is not naturally found, or in a replicon or vector where it is not naturally found, such as a bacterial plasmid or a viral vector. Chimeric DNA shall not be limited to DNA molecules which are replicable in a host, but shall also mean to comprise DNA capable of being ligated into a replicon, for instance by virtue of specific adaptor sequences, physically linked to the open reading frame according to the invention. The open reading frame may or may not be linked to its natural upstream and downstream regulatory elements.

The open reading frame may be derived from a genomic library. In this latter it may contain one or more introns separating the exons making up the open reading frame that encodes a protein according to the invention. The open reading frame may also be encoded by one uninterrupted exon, or by a cDNA to the mRNA encoding a protein according to the invention. Open reading frames according to the invention also comprise those in which one or more introns have been

artificially removed or added. Each of these variants is embraced by the present invention.

Also part of the invention are chimeric DNA sequences coding for an antifungal protein which comprise one or more of the EST-sequences  
5 shown in SEQ ID NO's: 21 to 48. As can be derived from the sequence listings these EST's for which no function was hitherto known share a considerable homology with the DNA sequence coding for the proteins isolated from *Helianthus* and *Lactuca*.

Another part of the invention is formed by the intrinsic  
10 activity of the proteins of the invention. They have been found to be carbohydrate oxidases, capable of oxidating a large number of different mono- and di-saccharides. The substrate specificity resembles the specificity of the enzyme hexose oxidase (EC 1.1.3.5), also known as D-hexose: oxygen 1-oxidoreductase. They have also been  
15 shown able to oxidise a purified mixture of fungal (*Rhizoctonia*-derived) cell wall components. It is believed that this oxidative capacity confers the antifungal properties to the proteins. In literature there is one example of an antifungal oxidase, the glucose oxidase from the fungus *Aspergillus* (WO 95/14784). The proteins of  
20 this invention, however, show a broader substrate spectrum like hexose oxidase and have a lower Km for the substrate.

From homology searches it has been found that some parts of the amino acid sequence of the proteins of the invention are more conserved and are related to sequences commonly found in oxidases. The  
25 highest homology has been found with reticuline oxidase, which enzyme is known from the family of Papaveraceae (Facchini, P.J. et al., Plant Physiol. 112, 1669-1677, 1996).

In order to be capable of being expressed in a host cell a chimeric DNA according to the invention will usually be provided with  
30 regulatory elements enabling it to be recognised by the biochemical machinery of the host and allowing for the open reading frame to be transcribed and/or translated in the host. It will usually comprise a transcriptional initiation region which may be suitably derived from any gene capable of being expressed in the host cell of choice, as  
35 well as a translational initiation region for ribosome recognition and attachment. In eukaryotic cells, an expression cassette usually comprises in addition a transcriptional termination region located downstream of said open reading frame, allowing transcription to

terminate and polyadenylation of the primary transcript to occur. In addition, the codon usage may be adapted to accepted codon usage of the host of choice. The principles governing the expression of a chimeric DNA construct in a chosen host cell are commonly understood  
5 by those of ordinary skill in the art and the construction of expressible chimeric DNA constructs is now routine for any sort of host cell, be it prokaryotic or eukaryotic.

In order for the open reading frame to be maintained in a host cell it will usually be provided in the form of a replicon comprising  
10 said open reading frame according to the invention linked to DNA which is recognised and replicated by the chosen host cell. Accordingly, the selection of the replicon is determined largely by the host cell of choice. Such principles as govern the selection of suitable replicons for a particular chosen host are well within the realm of the ordinary  
15 skilled person in the art.

A special type of replicon is one capable of transferring itself, or a part thereof, to another host cell, such as a plant cell, thereby co-transferring the open reading frame according to the invention to said plant cell. Replicons with such capability are  
20 herein referred to as vectors. An example of such vector is a Ti-plasmid vector which, when present in a suitable host, such as *Agrobacterium tumefaciens*, is capable of transferring part of itself, the so-called T-region, to a plant cell. Different types of Ti-plasmid vectors (vide: EP 0 116 718 B1) are now routinely being used to  
25 transfer chimeric DNA sequences into plant cells, or protoplasts, from which new plants may be generated which stably incorporate said chimeric DNA in their genomes. A particularly preferred form of Ti-plasmid vectors are the so-called binary vectors as claimed in (EP 0 120 516 B1 and US 4,940,838). Other suitable vectors, which may be  
30 used to introduce DNA according to the invention into a plant host, may be selected from the viral vectors, e.g. non-integrative plant viral vectors, such as derivable from the double stranded plant viruses (e.g. CaMV) and single stranded viruses, gemini viruses and the like. The use of such vectors may be advantageous, particularly  
35 when it is difficult to stably transform the plant host. Such may be the case with woody species, especially trees and vines.

The expression "host cells incorporating a chimeric DNA sequence according to the invention in their genome" shall mean to comprise



cells, as well as multicellular organisms comprising such cells, or essentially consisting of such cells, which stably incorporate said chimeric DNA into their genome thereby maintaining the chimeric DNA, and preferably transmitting a copy of such chimeric DNA to progeny cells, be it through mitosis or meiosis. According to a preferred embodiment of the invention plants are provided, which essentially consist of cells which incorporate one or more copies of said chimeric DNA into their genome, and which are capable of transmitting a copy or copies to their progeny, preferably in a Mendelian fashion. By virtue of the transcription and translation of the chimeric DNA according to the invention in some or all of the plant's cells, those cells that produce the antifungal protein will show enhanced resistance to fungal infections, especially to *Phytophthora* infections. Although the principles as indicated above govern transcription of DNA in plant cells are not always understood, the creation of chimeric DNA capable of being expressed in substantially a constitutive fashion, that is, in substantially most cell types of the plant and substantially without serious temporal and/or developmental restrictions, is now routine. Transcription initiation regions routinely in use for that purpose are promoters obtainable from the cauliflower mosaic virus, notably the 35S RNA and 19S RNA transcript promoters and the so-called T-DNA promoters of *Agrobacterium tumefaciens*, in particular to be mentioned are the nopaline synthase promoter, octopine synthase promoter (as disclosed in EP 0 122 791 B1) and the mannopine synthase promoter. In addition plant promoters may be used, which may be substantially constitutive, such as the rice actin gene promoter, or e.g. organ-specific, such as the root-specific promoter. Alternatively, pathogen-inducible promoters may be used such as the PRP1 promoter (also named *gst1* promoter) obtainable from potato (Martini N. et al. (1993), Mol. Gen. Genet. 263, 179-186). The choice of the promoter is not essential, although it must be said that constitutive high-level promoters are slightly preferred. It is further known that duplication of certain elements, so-called enhancers, may considerably enhance the expression level of the DNA under its regime (vide for instance: Kay R. et al. (1987), Science 236, 1299-1302: the duplication of the sequence between -343 and -90 of the CaMV 35S promoter increases the activity of that promoter). In addition to the 35S promoter, singly or doubly enhanced, examples of

high-level promoters are the light-inducible ribulose biphosphate carboxylase small subunit (*rbcSSU*) promoter and the chlorophyll a/b binding protein (*Cab*) promoter. Also envisaged by the present invention are hybrid promoters, which comprise elements of different promoter regions physically linked. A well known example thereof is the so-called CaMV enhanced mannopine synthase promoter (US Patent 5,106,739), which comprises elements of the mannopine synthase promoter linked to the CaMV enhancer.

As regards the necessity of a transcriptional terminator region, it is generally believed that such a region enhances the reliability as well as the efficiency of transcription in plant cells. Use thereof is therefore strongly preferred in the context of the present invention.

As regards the applicability of the invention in different plant species, it has to be mentioned that one particular embodiment of the invention is merely illustrated with transgenic tomato and tobacco plants as an example, the actual applicability being in fact not limited to these plant species. Any plant species that is subject to some form of fungal attack, in particular by Oomycetes such as *Phytophthora infestans*, may be treated with proteins according to the invention, or preferably, be provided with a chimeric DNA sequence according to the invention, allowing the protein to be produced in some or all of the plant's cells.

Although some of the embodiments of the invention may not be practicable at present, e.g. because some plant species are as yet recalcitrant to genetic transformation, the practicing of the invention in such plant species is merely a matter of time and not a matter of principle, because the amenability to genetic transformation as such is of no relevance to the underlying embodiment of the invention.

Transformation of plant species is now routine for an impressive number of plant species, including both the *Dicotyledoneae* as well as the *Monocotyledoneae*. In principle any transformation method may be used to introduce chimeric DNA according to the invention into a suitable ancestor cell, as long as the cells are capable of being regenerated into whole plants. Methods may suitably be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., 1982, *Nature* 296, 72-74; Negrutiu I. et al, June 1987, *Plant Mol.*

Biol. 8, 363-373), electroporation of protoplasts (Shillito R.D. et al., 1985 Bio/Technol. 3, 1099-1102), microinjection into plant material (Crossway A. et al., 1986, Mol. Gen. Genet. 202, 179-185), (DNA or RNA-coated) particle bombardment of various plant material  
5 (Klein T.M. et al., 1987, Nature 327, 70), infection with (non-integrative) viruses and the like. A preferred method according to the invention comprises *Agrobacterium*-mediated DNA transfer. Especially preferred is the use of the so-called binary vector technology as disclosed in EP A 120 516 and U.S. Patent 4,940,838).

10 Tomato transformation is preferably done essentially as described by Van Roekel et al. (Van Roekel, J.S.C., Damm, B., Melchers, L.S., Hoekema, A. (1993). Factors influencing transformation frequency of tomato (*Lycopersicon esculentum*). Plant Cell Reports, 12, 644-647). Potato transformation is preferably done essentially as described by

15 Hoekema et al. (Hoekema, A., Huisman, M.J., Molendijk, L., van den Elzen, P.J.M., and Cornelissen, B.J.C. (1989). The genetic engineering of two commercial potato cultivars for resistance to potato virus X. — Bio/Technology 7, 273-278).

Generally, after transformation plant cells or cell groupings are  
20 selected for the presence of one or more markers which are encoded by plant expressible genes co-transferred with the nucleic acid sequence encoding the protein according to the invention, whereafter the transformed material is regenerated into a whole plant.

Although considered somewhat more recalcitrant towards genetic  
25 transformation, monocotyledonous plants are amenable to transformation and fertile transgenic plants can be regenerated from transformed cells or embryos, or other plant material. Presently, preferred methods for transformation of monocots are microprojectile bombardment of embryos, explants or suspension cells, and direct DNA uptake or  
30 electroporation (Shimamoto, et al, 1989, Nature 338, 274-276).

Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus* bar-gene, which encodes phosphinothricin acetyltransferase (an enzyme which inactivates the herbicide phosphinothricin), into embryogenic cells of a maize suspension  
35 culture by microprojectile bombardment (Gordon-Kamm, 1990, Plant Cell, 2, 603-618). The introduction of genetic material into aleurone protoplasts of other monocot crops such as wheat and barley has been reported (Lee, 1989, Plant Mol. Biol. 13, 21-30). Wheat plants have

been regenerated from embryogenic suspension culture by selecting only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, 1990 Bio/Technol. 8, 429-434). The combination with transformation systems  
5 for these crops enables the application of the present invention to monocots.

Monocotyledonous plants, including commercially important crops such as rice and corn are also amenable to DNA transfer by *Agrobacterium* strains (vide WO 94/00977; EP 0 159 418 B1; Gould J, Michael D, Hasegawa O, Ulian EC, Peterson G, Smith RH, (1991) Plant.  
10 Physiol. 95, 426-434).

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the chimeric DNA according to the invention, copy number  
15 and/or genomic organization. In addition, or alternatively, expression levels of the newly introduced DNA may be undertaken, using Northern and/or Western analysis, techniques well known to persons having ordinary skill in the art. After the initial analysis, which is optional, transformed plants showing the desired copy number and  
20 expression level of the newly introduced chimeric DNA according to the invention may be tested for resistance levels against a pathogen susceptible to the protein according to the invention, such as *Phytophthora infestans*. Alternatively, the selected plants may be subjected to another round of transformation, for instance to  
25 introduce further genes, such as genes encoding chitinases, glucanases, osmotins, magainins or the like, in order to enhance resistance levels, or broaden the resistance to other fungi found not to be susceptible to the protein according to the invention in an in vitro assay as described herein.

30 Other evaluations may include the testing of fungal resistance under field conditions, checking fertility, yield, and other characteristics. Such testing is now routinely performed by persons having ordinary skill in the art.

Following such evaluations, the transformed plants may be grown  
35 directly, but usually they may be used as parental lines in the breeding of new varieties or in the creation of hybrids and the like.

Many plant proteins exhibit antifungal effects, some however do not do so as such, but yield a significant synergistic antifungal

effect if used in combination with other plant proteins. In European Patent Application 440 304 A1 it was disclosed that simultaneous relative over-expression of a plant expressible glucanase gene in conjunction with a basic chitinase from tobacco in transgenic plants results in a higher level of resistance to fungi than in plants expressing a plant expressible class-I chitinase alone.

Both chitinases, glucanases, osmotins, magainins and the new antifungal protein according to the invention accumulate in infected plant tissues upon an incompatible pathogen-plant interaction. From this observation and the fact that several proteins are found to synergise each others antifungal effects, we envision, that the antifungal protein according to the invention may be suitably used in conjunction with other proteins that are associated with pathogen resistance.

Examples of proteins that may be used in combination with the proteins according to the invention include, but are not limited to,  $\beta$ -1,3-glucanases and chitinases which are obtainable from barley (Swegle M. et al., 1989, Plant Mol. Biol. 12, 403-412; Balance G.M. et al., 1976, Can. J. Plant Sci. 56, 459-466 ; Hoj P.B. et al., 1988, FEBS Lett. 230, 67-71; Hoj P.B. et al., 1989, Plant Mol. Biol. 13, 31-42 1989), bean (Boller T. et al., 1983, Planta 157, 22-31; Broglie K.E. et al. 1986, Proc. Natl. Acad. Sci. USA 83, 6820-6824; Vögeli U. et al., 1988 Planta 174, 364-372); Mauch F. & Staehelin L.A., 1989, Plant Cell 1, 447-457); cucumber (Motraux J.P. & Boller T. (1986), Physiol. Mol. Plant Pathol. 28, 161-169); leek (Spanu P. et al., 1989, Planta 177, 447-455); maize (Nasser W. et al., 1988, Plant Mol. Biol. 11, 529-538), oat (Fink W. et al., 1988, Plant Physiol. 88, 270-275), pea (Mauch F. et al. 1984, Plant Physiol. 76, 607-611; Mauch F. et al., 1988, Plant Physiol. 87, 325-333), poplar (Parsons, T.J. et al, 1989, Proc. Natl. Acad. Sci. USA 86, 7895-7899), potato (Gaynor J.J. 1988, Nucl. Acids Res. 16, 5210; Kombrink E. et al. 1988, Proc. Natl. Acad. Sci. USA 85, 782-786; Laflamme D. and Roxby R., 1989, Plant Mol. Biol. 13, 249-250), tobacco (e.g. Legrand M. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 6750-6754; Shinshi H. et al. 1987, Proc. Natl. Acad. Sci. USA 84, 89-93), tomato (Joosten M.H.A. & De Wit P.J.G.M. 1989, Plant Physiol. 89, 945-951), wheat (Molano J. et al., 1979, J. Biol. Chem. 254, 4901-4907), and the like.

To obtain transgenic plants capable of constitutively expressing more than one chimeric gene, a number of alternatives are available including the following:

- A. The use of DNA, e.g. a T-DNA on a binary plasmid, with a number of  
5 modified genes physically coupled to a selectable marker gene. The advantage of this method is that the chimeric genes are physically coupled and therefore migrate as a single Mendelian locus.
- B. Cross-pollination of transgenic plants each already capable of  
10 expressing one or more chimeric genes, preferably coupled to a selectable marker gene, with pollen from a transgenic plant which contains one or more chimeric genes coupled to another selectable marker. Afterwards the seed, which is obtained by this crossing, maybe selected on the basis of the presence of the two selectable markers, or on the basis of the presence of the chimeric genes themselves. The  
15 plants obtained from the selected seeds can afterwards be used for further crossing. In principle the chimeric genes are not on a single locus and the genes may therefore segregate as independent loci.
- C. The use of a number of a plurality chimeric DNA molecules, e.g. plasmids, each having one or more chimeric genes and a selectable  
20 marker. If the frequency of co-transformation is high, then selection on the basis of only one marker is sufficient. In other cases, the selection on the basis of more than one marker is preferred.
- D. Consecutive transformation of transgenic plants already containing a first, second, (etc), chimeric gene with new chimeric DNA,  
25 optionally comprising a selectable marker gene. As in method B, the chimeric genes are in principle not on a single locus and the chimeric genes may therefore segregate as independent loci.
- E. Combinations of the above mentioned strategies.

The actual strategy may depend on several considerations as  
30 maybe easily determined such as the purpose of the parental lines (direct growing, use in a breeding programme, use to produce hybrids) but is not critical with respect to the described invention.

In this context it should be emphasised that plants already containing chimeric DNA capable of encoding antifungal proteins may  
35 form a suitable genetic background for introducing chimeric DNA according to the invention, for instance in order to enhance resistance levels, or broaden the resistance. The cloning of other genes corresponding to proteins that can suitably be used in

combination with DNA, and the obtention of transgenic plants, capable of relatively over-expressing same, as well as the assessment of their effect on pathogen resistance in planta, is now within the scope of the ordinary skilled person in the art.

5       The obtention of transgenic plants capable of expressing, or relatively over-expressing, proteins according to the invention is a preferred method for counteracting the damages caused by fungi, such as Oomycetes like *Phytophthora infestans*, as will be clear from the above description. However, the invention is not limited thereto. The  
10       invention clearly envisions also the use of the proteins according to the invention as such, preferably in the form of a fungicidal composition. Fungicidal composition include those in which the protein is formulated as such, but also in the form of host cells, such as bacterial cells, capable of producing the protein thereby causing the  
15       pathogen to be contacted with the protein. Suitable host cells may for instance be selected from harmless bacteria and fungi, preferably those that are capable of colonising roots and/or leaves of plants. Example of bacterial hosts that may be used in a method according to the invention are strains of *Agrobacterium*, *Arthrobacter*,  
20       *Azospyrillum*, *Pseudomonas*, *Rhizobacterium*, and the like, optionally after having been made suitable for that purpose.

Compositions containing antifungal proteins according to the invention may comprise in addition thereto, osmotin-like proteins as defined in WO91/18984. Independently, the invention provides  
25       antifungal compositions which further comprise inhibitory agents such as classical fungal antibiotics, SAFPs and chemical fungicides such as polyoxines, nikkomycines, carboxymides, aromatic carbohydrates, carboxines, morpholines, inhibitors of sterol biosynthesis, organophosphorus compounds, enzymes such as glucanases, chitinases,  
30       lysozymes and the like. Either per se, or in combination with other active constituents, the antifungal protein of the invention should be applied in concentrations between 1 ng/ml and 1 mg/ml, preferably between 2 ng/ml and 0.1 mg/ml, within pH boundaries of 3.0 and 9.0. In general it is desired to use buffered preparations, e.g. phosphate  
35       buffers between 1mM and 1M, preferably between 10 mM and 100mM, in particular between 15 and 50 mM, whereby in case of low buffer concentrations it is desired to add a salt to increase ionic strength, preferably NaCl in concentrations between 1 mM and 1M, preferably 10

mM and 100 mM.

- Plants, or parts thereof, which relatively over-express a protein according to the invention, including plant varieties, with improved resistance against fungal diseases, especially diseases
- 5 caused by Oomycetes like *Phytophthora* and *Pythium* may be grown in the field, in the greenhouse, or at home or elsewhere. Plants or edible parts thereof may be used for animal feed or human consumption, or may be processed for food, feed or other purposes in any form of
- 10 agriculture or industry. Agriculture shall mean to include horticulture, arboriculture, flower culture, and the like. Industries which may benefit from plant material according to the invention include but are not limited to the pharmaceutical industry, the paper and pulp manufacturing industry, sugar manufacturing industry, feed and food industry, enzyme manufacturers and the like.
- 15 The advantages of the plants, or parts thereof, according to the invention are the decreased need for fungicide treatment, thus lowering costs of material, labour, and environmental pollution, or prolonging shelf-life of products (e.g. fruit, seed, and the like) of such plants. Plants for the purpose of this invention shall mean
- 20 multicellular organisms capable of photosynthesis, and subject to some form of fungal disease. They shall at least include angiosperms as well as gymnosperms, monocotyledonous as well as dicotyledonous plants.

- The phrase "plants which relatively over-express a protein"
- 25 shall mean plants which contain cells expressing a transgene-encoded protein which is either not naturally present in said plant, or if it is present by virtue of an endogenous gene encoding an identical protein, not in the same quantity, or not in the same cells, compartments of cells, tissues or organs of the plant. It is known for
- 30 instance that proteins which normally accumulate intracellularly may be targeted to the apoplastic space.

- According to another aspect of the invention the regulatory region of a plant gene coding for the antifungal protein of the invention may be used to express other heterologous sequences under
- 35 the control thereof. The use of a regulatory element of at least 1000 bp directly upstream of the gene coding region is sufficient for obtaining expression of any heterologous sequence.



Heterologous sequences in this respect means gene regions not naturally associated to said regulatory region, and they comprise both different gene coding regions, as well as antisense gene regions. Heterologous coding sequences that may be advantageously expressed in the vascular tissue comprise those coding for antipathogenic proteins, e.g. insecticidal, bactericidal, fungicidal, and nematocidal proteins. In such a strategy it may prove exceptionally advantageous to select a protein with activity against a pathogen or pest which has a preference for phloem as source of nutrients (e.g. aphids), or as entrance to invade the plant. Examples are extensin, lectin, or lipoxidase against aphids (See WO93/04177). Assuming that the regulatory region according to the invention is active in xylem, antifungal proteins may be expressed under the control of said regulatory region to combat *Fusarium*, *Verticillium*, *Alternaria* and *Ceratocystus* species.

The use of the regulatory region according to the invention may also be used advantageously to regulate or control phloem transport processes. Numerous other applications will readily occur to those of skill in the art.

The expression of part of (part of) an endogenous gene in the antisense orientation (such as disclosed in EP 0 233 399 A), can effectively down-regulate expression of said endogenous gene, with interesting applications. Moreover, the gene encoding the antifungal protein according to the invention itself may be down-regulated using the antisense approach which may help establishing the nature and function of the protein. The regions responsible for tissue-specific expression may be unravelled further using the GUS-marker in a way analogous to the way illustrated herein.

The following state of the art may be taken into consideration, especially as illustrating the general level of skill in the art to which this invention pertains.

EP-A 392 225 A2; EP-A 440 304 A1; EP-A 460 753 A2; WO90/07001 A1; US Patent 4,940,840.

Yet another part of the invention is directed at the production of a novel oxidative enzyme, capable of oxidising carbohydrates even at low concentrations due to its low  $K_m$ . Most specifically hexoses are the substrate of the enzymatic activity although also other sugars are

affected to some lesser extent. The enzymes can be isolated from the sources in which they naturally occur (according to the method described in this invention) or they can be isolated from plants or other organisms transformed with an expressible gene encoding the protein. These oxidases can be used in industrial processes for the oxidation of carbohydrates, such as glucose, mannose, galactose, cellobiose, maltose and lactose.

#### Evaluation of transgenic plants

Subsequently transformed plants are evaluated for the presence of the desired properties and/or the extent to which the desired properties are expressed. A first evaluation may include the level of expression of the newly introduced genes, the level of fungal resistance of the transformed plants, stable heritability of the desired properties, field trials and the like.

Secondly, if desirable, the transformed plants can be crossbred with other varieties, for instance varieties of higher commercial value or varieties in which other desired characteristics have already been introduced, or used for the creation of hybrid seeds, or be subject to another round of transformation and the like.

#### Synergy

The combination of one of the antifungal protein according to the instant invention and other antifungal proteins of plant or microbial source are predicted to show a drastic synergistic antifungal effect. Similar synergistic antifungal effects were shown if combinations of antifungal CBPs or Chi-V are combined with either  $\beta$ -1,3-glucanases or chitinases from other plant origins. Apparently, the synergizing effect of combinations of pathogen induced proteins is a more general phenomenon that has important consequences for the engineering of fungal resistant plants.

Plants, or parts thereof of commercial interest, with improved resistance against phytopathogenic fungi can be grown in the field or in greenhouses, and subsequently be used for animal feed, direct consumption by humans, for prolonged storage, used in food- or other industrial processing, and the like. The advantages of the plants, or parts thereof, according to the invention are the decreased need for

fungicide treatment, thus lowering costs of material, labour, and environmental pollution, or prolonged shelf-life of products (e.g. fruit, seed, and the like) of such plants.

5

#### EXPERIMENTAL PART

Standard methods for the isolation, manipulation and amplification of DNA, as well as suitable vectors for replication of recombinant DNA, suitable bacterium strains, selection markers, media and the like are described for instance in Maniatis et al., molecular cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press; DNA Cloning: Volumes I and II (D.N. Glover ed. 1985); and in: From Genes To Clones (E.-L. Winnacker ed. 1987).

#### In vitro antifungal assay

15 All fungi were cultured on potato dextrose agar (Difco) at 25°C, except *Botrytis cinerea* and *Phoma lingam* which were grown on oat meal agar (Difco) at 25°C. *Phytophthora infestans* was grown on rye agar at 18°C in the dark (Caten and Jinks, 1968). *Botrytis cinerea* and *Phoma lingam* were cultivated under UV. Spores of sporulating fungi were  
20 harvested by flooding the agar plates with water. The spore concentration was adjusted to 10,000 sp/mL. In the case of *Rhizoctonia solani* and *Pythium ultimum* liquid shake cultures were grown in potato dextrose broth at 25°C. To prepare inoculum from these shake cultures, mycelium was harvested and vortexed for 1 minute. After passage  
25 through a fine sieve, inoculum density was adjusted to 2500 - 5000 fragments, of 1 to 3 cells each, per mL.

In case of sporulating fungi, all were tested both with and without pregerminating the spores before application of the protein samples. In case of non-sporulating fungi, hyphal fragments were used.

30 The antifungal activity was monitored during purification in a microtiter plate assay using the fungi *Phytophthora infestans* and *Pythium ultimum* according to Woloshuk et al., 1991 or using other fungi in a similar way. In each well of a 24-well microtiter dish 250 µl potato dextrose agar (PDA) was pipetted. Fungal spores in the case  
35 of e.g. *Phytophthora infestans* and hyphal fragments in the case of e.g. *Pythium ultimum* were suspended in water and 400-600 spores or 200 fragments in 50 µl were added to the wells. Subsequently 100 µl filter sterilized (0.22 µm filter) protein solution (in 50 mM MES, pH 6.0)

was added. Microtiter dishes were wrapped with Parafilm and incubated at room temperature. At several timepoints after the initiation of incubation the fungus was monitored microscopically for effects of the added protein. After 2-3 days the mycelium of the growing fungus in the wells was stained with lactophenol cotton blue and the extent of growth was estimated.

GI: growth inhibition; a scale of 0 - 4 is used, 0 = no visible inhibition, 1 = weak inhibition (0 to 30%) inhibition, 2 = moderate (30 to 60%) inhibition, 3 = strong (60 to 90%) inhibition, 4 = very strong (100%) inhibition.

#### EXAMPLE 1

##### **Purification of an antifungal protein MS59 from sunflower induced with salicylic acid**

Leaves of 7 to 8 weeks old sunflower (*Helianthus annuus* cv. zebulon) plants were sprayed daily for 5 times with 10 mM sodium salicylate. After 3 hours the plants were extensively rinsed with water to remove the sodium salicylate. Three days after the final spray, leaves (400 gram) were harvested into liquid nitrogen and homogenized at 4°C in 500 ml 0.5 M NaOAc pH5.2, and 4 gram active carbon, using a Waring blender. The homogenate was filtered over four layers of cheese cloth and subsequently the filtrate was centrifuged for 50 minutes at 20,000 g at 4°C and desalted by passage through a Sephadex G25 column (medium course; Pharmacia), length 60 cm, diameter 11.5 cm, equilibrated in 40 mM NaOAc pH5.2. The desalted protein solution was stored overnight at 4°C and subsequently centrifuged for 45 minutes at 20,000 g at 4°C. The supernatant was passed through a S-sephadex (Fast-flow, Pharmacia) column, length 5 cm, diameter 5 cm, which was equilibrated with 40 mM NaOAc pH 5.2. The column was washed with the above mentioned buffer (flow rate 400 to 500 ml/hr) until the OD<sub>280</sub> dropped to zero. The bound proteins were eluted using 400 mM NaCl in 200 ml of the above mentioned buffer.

After dialysis against 50 mM MES pH 6.0 the eluate was analyzed for antifungal activity. Antifungal activity was monitored in a microtiter plate assay using the fungus *Phytophthora infestans* and *Pythium ultimum*. See above for details concerning *in vitro* assaying. Subsequently, cationexchange chromatography was reapplied whereby the eluate was passed through an FPLC Mono-S HR 5/5 (Pharmacia) and eluted

with a linear gradient from 0 to 400 mM NaCl. All fractions were analyzed by electrophoresis (Laemmli (1970), Nature 227:680-685) using a 12.5% polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS), using prestained molecular weight markers (15-105 kDa) as reference. Additionally, of all fractions antifungal activity towards *Phytophthora infestans* and *Pythium ultimum* was monitored. Antifungal activity eluted from the column between 45-60 mM NaCl and in all active fractions a 59 kD band was visible. Fractions containing the antifungal activity were pooled and dialysed to 1 M ammonium sulphate in 50 mM potassium phosphate, pH 7. The pool was subjected to hydrophobic interaction chromatography, whereby the sample was applied to an FPLC Phenyl Superose HR 5/5 (Pharmacia) equilibrated in the same buffer and eluted with a linear decreasing gradient from 1 to 0 M ammonium sulphate in 50 mM potassium phosphate, pH 7. As above again all fractions were analyzed on SDS-PAGE and monitored for antifungal activity. Also the pool of proteins not capable of binding to this column (Flow Through, FT) was thus analyzed at the conditions chosen here. Antifungal activity was present most abundantly in the FT and secondly also in the fractions eluting between 0.76 and 0.45 mM ammonium sulphate. In both cases a 59 kD protein was visible on SDS-PAGE. FT and the gradient fractions were separately dialysed to 50 mM MES, 0.2 M NaCl and separately chromatographed on a FPLC Superdex 75 HR 10/30 column (Pharmacia) equilibrated to the same buffer. Proteins elute from this column according to their molecular size. In both cases again the presence of a 59 kD protein coincided with antifungal activity towards *Phytophthora infestans* and *Pythium ultimum* as judged from SDS-PAGE and *in vitro* antifungal assays. The 59 kD protein present in the FT of the hydrophobic interaction column was most abundant and termed MS59 and its purification is visualized in Figure 1. Results of its separation over the gel filtration column and subsequent analysis both on SDS-PAGE and on *Phytophthora infestans* is shown in Figure 2. Several characteristics (antifungal activity, chromatographical properties, molecular mass) of the gradient protein and MS59 indicate that the two proteins are very similar.

To characterize MS59 further its amino acid sequence was partially determined. Therefore, MS59 was separated in the presence of 0.1 mM thioglycolate in the upper reservoir buffer and SDS on a 12.5% polyacrylamide gel, which was prerun for 2 hours at 50 V with 0.05 mM

glutathione in the upper reservoir buffer. The gel was stained with 5% (w/v) Serva Blue G in 45% (v/v) methanol and 10% acetic acid for 30 minutes and destained in 20% (v/v) acetic acid for 30 minutes and the 59 kDa band was cut out and sequenced using Edman degradation on an Applied Biosystems 477A protein sequencer according to the protocol provided by the manufacturer. N-terminal amino acid sequencing of MS59 revealed that the N-terminus was blocked. To obtain internal sequences, MS59 was digested with trypsin. Trypsin cleaves protein at arginine and lysine residues. The digestion products were separated on a reversed-phase column and analyzed by Edman degradation. Two tryptic fragments were sequenced: Pep1 and Pep2. Of Pep1 25 amino acid residues were identified: S-I-N-V-D-I-E-Q-E-T-A-W-V-Q-A-G-A-T-L-G-E-V-Y-Y-R (SEQ ID NO: 1).

The amino acid sequence is given using the one-letter code. Of Pep2 a further 25 amino acid residues were identified: D-P-S-F-P-I-T-G-E-V-Y-T-P-G-(?)-S-S-F-P-T-V-L-Q-N-Y (SEQ ID NO: 2). The amino acid residue between brackets could not be identified unambiguously.

20

#### EXAMPLE 2

##### **Elution of antifungal protein from native PAGE and subsequent testing**

It is obvious from Figures 1 and 2 that MS59 is not completely pure. To further ensure that indeed the 59 kDa protein is responsible for the observed antifungal activity, the fraction containing the peak amount of 59 kDa was electrophoresed on a native gel, using the same system as described above however without SDS and without boiling the samples before loading. The gel lane was sliced in 0.5 cm horizontal pieces and each piece was eluted individually for 48 hours in 50 mM Mes, pH 6. After centrifugation the resulting supernatant was analyzed both on SDS-PAGE and *in vitro* for antifungal activity. Results are shown in Figure 3. Only in those fractions containing MS59, was antifungal activity observed against *Phytophthora infestans* and *Pythium ultimum*.

EXAMPLE 3**In vitro antifungal assays on non-Oocymetes**

In vitro fungal assays were performed as described in the general experimental part. As positive control *Phytophthora infestans* was tested. The peak of MS59 is located in fraction 4. Results are shown in Table 1.

**Table 1. Antifungal effects of MS59 containing fractions from Mono-S, pH 6**

10										
	fungus	spore	fraction number							
		stage *)	1	2	3	4	5	6	7/8	
	Fusarium oxysporum	spore	0	0	0	0	0	0	0	
15		germl.	2	2	2	2	3	3.5	3.5	
	Fusarium solani	spore	0	0	0	0	0	0	0	
	Phytophthora infestans	spore	0	2	2	4	3.5	2	1	
	Phytophthora nicotianae	hyph	0	1	2	4	4	2	1	
	Phytophthora cactorum	hyph	0	0	2	4	4	1	1	
20	Pythium ultimum	hyph	0	0	0	4	4	0	0	
	Pythium sylvaticum	hyph	0	0	0	2	1	0	0	
	Pythium paroecandrum	hyph	0	0	0	2	2	0	0	

\*) spore = no pregermination, germl = germination until the germ tube is 3-5 times the length of the spore, hyph. = hyphal fragments were used as starting inoculum.

GI: growth inhibition; a scale of 0 - 4 is used, 0 = no visible growth inhibition, 1 = weak (0 to 30%) inhibition, 2 = moderate (30 to 60%) inhibition, 3 = strong (60 to 90%) inhibition, 4 = very strong (100%) inhibition.

As can be seen *Phytophthora* and *Pythium* spp., appeared very sensitive to MS59.

EXAMPLE 4

**Purification of an antifungal protein WL64 from lettuce  
induced with salicylic acid**

Leaves of 7 to 8 weeks old lettuce (*Lactuca sativa* cv. Lollo  
5 bionda) plants were sprayed daily with 10 mM salicylate for 4 days.  
After two hours the plants were extensively rinsed with water to  
remove the sodium salicylate. On day 5, the leaves were harvested into  
liquid nitrogen and stored at -80°C until further use.

Lettuce leaves were thawed and homogenized at 4°C in 0.5M NaOAc  
10 pH 5.2, 0.1%  $\beta$ -mercaptoethanol (lettuce : buffer = 1: 1.5 (w/v)), and  
10 grams active carbon per kg leaves, using a Waring blender. The  
homogenate was centrifuged for 60 minutes at 9,000 g at 4°C. The  
supernatant was subsequently filtered over 10 layers of cheese cloth.  
The filtrate was brought to 40% saturation with ammonium sulphate and  
15 centrifuged for 30 minutes at 9,000 g. The resulting supernatant,  
containing 85% of protein and >95% of antifungal activity relative to  
the crude homogenate, was subjected to hydrophobic interaction  
chromatography.

The supernatant was filtered over a paper filter and applied to  
20 a phenyl-sepharose 6FF High sub column (Pharmacia, 100 ml bed volume  
in a Pharmacia XK 50/20 column) pre-equilibrated with 40% (1.45M)  
ammonium sulphate in 50mM potassium-phosphate buffer, pH 6.0 (referred  
to as buffer A) at a flow rate of 10 ml/min or less. The column was  
washed with at least 10 column volumes of buffer A after which bound  
25 protein was eluted with a decreasing salt gradient from 100% buffer A  
to 20% buffer A (50mM KPi pH 6.0 as buffer B) over a period of 40 min  
at a flow rate of 10 ml/min, followed by a linear decreasing gradient  
from 20% A to 0% A (=100% B) over a period of 30 min at the same flow  
rate. The column was washed for another 45 min with buffer B, after  
30 which the elution was completed. One-minute fractions were collected  
(10 ml/fraction). Fractions 40-75 (called the HIC-peak) contained  
antifungal activity.

The pooled fractions were concentrated (using a stirred flow  
cell and a YM 30 kDa membrane (Amicon)) and subsequently 15 times  
35 diluted with 25mM sodium acetate, pH 4.5. This solution was applied to  
a pre-packed Source S column (16/20, Pharmacia) with a flow rate of 10  
ml/min. After washing of the column with 5 column volumes of said  
buffer, protein was eluted from the column with an increasing NaCl



gradient (0-0.4M NaCl in 25mM NaOAc, pH 4.5) over a 60 min period, 2.5 ml/min, 1 min fractions. Fractions were collected in 250µl 1M potassium phosphate, pH 7.0, in order to neutralize the relatively acidic NaOAc buffer. The fractions containing antifungal activity (fractions 25-45 (0.2-0.3M NaCl)) were pooled and are referred to as the Source S-peak.

The Source S-peak was concentrated and buffer exchanged to 25mM NaOAc, pH 4.5, resulting in a fraction of about 10 ml, and subjected to cation-exchange chromatography using a Mono S column (5/5, Pharmacia). The column is eluted with the following NaCl gradients (NaCl in 25mM NaOAc, pH 4.5): 0-5 min, 0-0.1M NaCl; 5-20 min, 0.1-0.16M NaCl; 20-21 min, 0.16-0.25M NaCl; 21-31 min, 0.25M NaCl; 31-32 min, 0.25-1.0M NaCl, followed by 1.0M NaCl for 10 min after which the elution is completed. The antifungal activity eluted from the column during the 0.25M NaCl step (usually fractions 22-30; the Mono-S peak). Flow-rate 1 ml/min, 1ml fractions, collected in 100µl 1M potassium phosphate, pH 7.0.

The Mono S-peak was concentrated to about 0.5-1.0 ml and subjected to gelfiltration chromatography (Superdex 75, 10/30, Pharmacia), with 200mM NaCl in 50mM potassium phosphate, pH 7.0 as the running buffer. The sample volume was 200 µl; flow rate 0.5 ml/min; 0.5 ml/fraction. The antifungal activity elutes from the column at the position of the 66 kDa marker. Comparison of the active fractions (SD 75 peak) with the protein pattern on SDS-PAGE reveals a 64 kDa protein as the most likely candidate for the lettuce-antifungal protein (Figs. 6A-C). This protein was named WL64.

The SD 75-peak was buffer-exchanged to pH 9.5 for chromatophocusing on a Mono P column (Pharmacia) according to the manufacturers instructions. All activity was found in the flow-through of the column (even in the case when the column was equilibrated to pH 11.0) although there was some separation (3 overlapping peaks in flow-through). The flow-rate was 0.5 ml/min; 0.5 ml/fraction. The fractions containing the anti-fungal activity were pooled and buffer-exchanged to 50mM MES, pH 6.0. Coomassie staining of the highest purified protein fraction after SDS-PAGE revealed about 6 protein bands of which two bands of 64 kDa and 55 kDa, were the most prominent ones (Fig. 7). The estimated relative amounts of both proteins in the final fraction was 1/6-1/8 for the 64 kDa protein and 1/2-1/3 for the 55 kDa

protein. Although on gel it is shown that this column clearly contributes to the purification of the 64 kDa protein, the specific activity, as well as the recovery of the protein in the pooled fractions dropped considerably (see table 2).

- 5 A representative purification procedure is summarized in table 2.

Table 2. Purification of WL 64

Sample or Column	Protein (mg)	Activity (GI-units)	Spec. act. (GI-u/mg)	Purifica- tion (x-fold)	Recovery (%)
Lettuce (1.54 kg)					
Extract	685				
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> sup	584	101250	173	1	100
HIC	174	44000	253	1.46	43
Source S	38.7	32400	837	4.84	32
Mono S	2.3	8960	3896	22.5	8.8
SD-75	0.452	8200	18142	105	8.1
Mono P	0.137	1752	12788	74	1.7

- 10 The activity is represented as growth inhibition units (GI-units). Four GI units represent the amount of protein that results in a growth inhibition of 100% in the in vitro assay as described in the general part of the Examples.

15

#### EXAMPLE 5

##### Elution of WL64 from native PAGE and subsequent testing

- Since WL64 was not completely pure, it was further investigated whether or not the 64 kDa protein was indeed responsible for the observed antifungal activity. The Mono P fraction containing the peak amount of antifungal activity was submitted to electrophoresis on a native 10% polyacrylamide gel under acidic conditions, in the absence of SDS and  $\beta$ -mercaptoethanol and without boiling. Two adjacent gel lanes were sliced in 0.3 cm horizontal pieces. One part was used

directly in the antifungal assay, the other part was subjected to SDS-PAGE under denaturing conditions. Growth inhibition clearly correlated to the 64 kDa protein and not to the 55 kDa protein.

5

**EXAMPLE 6****Glycosylation of WL64**

WL64, as well as the 55kDa protein are glycosylated as illustrated by binding to concanavalin A and by the DIG-Glycan-detection kit (Boehringer). Both proteins were not sensitive to glycopeptidase-F treatment, indicating that the glycosylation is probably O-linked.

**EXAMPLE 7****Amino acid sequencing of WL64**

For N-terminal amino acid sequencing an amount of 21 µg of purified protein (representing about 4µg WL64) was separated on a 7.5% polyacrylamide gel and was subsequently blotted onto PVDF membrane. The membrane was stained with 0.1% Serva Blue G in 45% methanol, 10% acetic acid for 5 minutes at room temperature and destained with 45% methanol, 10% acetic acid. The 64 kDa band was cut out and sequenced using Edman degradation on an Applied Biosystems 477A protein sequencer according to the protocol provided by the manufacturer.

For internal protein sequencing 105 µg of purified protein (representing about 20µg WL64) was separated on a 7.5% SDS-polyacrylamide gel. The gel was stained with 0.2% Serva Blue G in 20% methanol, 0.5% acetic acid for 20 min at room temperature and destained with 30% methanol at room temperature for about 1 hour. The 64 kDa band was cut out and the protein was subsequently digested with trypsin. The digestion products were separated on a reverse phase column and analyzed by Edman degradation.

Besides the N-terminal sequence (SEQ ID NO: 49), two tryptic fragments were sequenced (SEQ ID NO: 50 and SEQ ID NO: 51).

SEQ ID NO: 49: Thr-Ser-Thr-Ser-Ile-Ile-Asp-Arg-Phe-Thr-Gln-(Cys/Ser)-Leu-Asn-Asn-Arg-Ala-Asp-Pro-(Ser)-(Phe)-

SEQ ID NO: 50: (Ser)-Ile-(???) -Val-(Ser)-Ile-Glu-Asp-Glu-Thr-Ala-(Trp)-Val-Gln-Ala-Gly-Ala-Thr-Leu-Gly-Glu-Val-Tyr-(Tyr)-

SEQ ID NO: 51: Ala-Asp-Pro-Ser-Phe-Pro-Leu-Ser-Gly-Gln-Leu-Tyr-Thr-Pro-

The amino acid residues between brackets could not be identified unambiguously.

#### EXAMPLE 8

##### 5                    **Anti-fungal activity of MS59 and WL64**

Based on the sequence homology between MS59 and WL64, both proteins appear to be very related to each other. This might also be the case for their anti-fungal activity, as well as for their specific activities towards the respective fungi. This hypothesis was tested  
10 and the results are summarized in table 3.

**Table 3.      Anti-fungal activity of MS59 and WL64 <sup>1</sup>**

Pathogen	Amount of WL64 needed for complete inhibition (GI 4) (ngram per assay)	Amount of MS59 needed for complete inhibition (GI 4) (ngram per assay)
<i>Phytophthora infestans</i>	10	5
<i>Pythium ultimum</i>	10	5
<i>Rhizoctonia solani</i>	20	10
<i>Tanatephorus cucumeris</i>	20	n.t.
<i>Helicobasidium purpureum</i>	15	7.5
<i>Sclerotium cepivorum</i>	40	20
<i>Pichia pastoris</i>	n.t.	5
<i>Botrytis cinerea</i>	200	10

n.t. = not tested

15 <sup>1</sup> Antifungal assays were carried out as described in the general experimental part.

Note that the amounts of protein were estimated by means of Coomassie staining on SDS-PAGE gels, meaning that the amounts of protein depicted here are indicative, rather than absolute.

EXAMPLE 9**Oxidase activities**

A 50ml culture of *Rhizoctonia solani* in potato dextrose broth was extensively sonicated on ice and subsequently centrifuged at 3,000 g for 20 minutes at 4°C. The resulting supernatant was then centrifuged at 25,000 g for 1 hour. The pellet was washed twice with demineralized water and resuspended in 1 ml water containing 1.0% Triton X-100. In this way a fungal cell wall suspension was obtained.

Oxidase activity was measured utilizing the reagent 4-amino-antipyrine (4-AAP), based on Gallo, 1981 (Gallo, Methods in Enzymology, 71:665-668, 1981). A reaction volume of 500µl contained 50mM potassium phosphate buffer pH 7.0, 25µM FAD, 10mM NaN<sub>3</sub>, 0.01% Triton X-100, 6mM 2,4,6,tribromohydroxybenzoic acid, 2mM 4-AAP, and 10 units horseradish peroxidase. Hydrogenperoxide production was measured at 510nm. Known amounts of hydrogen peroxide were included for calibration.

WL64, as well as MS59, performed oxidase activities using the fungal cell wall suspension as substrate. Different substances were subsequently tested as possible substrates, a.o. some carbohydrates and amino acids (see example 10). Glucose, and other carbohydrates were found to serve as substrate for the oxidase activity of both MS59 and WL64.

Since MS59 and WL64 displayed carbohydrate and especially glucose oxidase activity it was investigated whether the fungal cell wall suspension could serve as a substrate for glucose oxidase (GOX) from *Aspergillus niger* (Sigma, G 2133). This was indeed the case. Kinetic studies showed that MS59, WL64 and GOX display Michaelis-Menten kinetics when glucose is used as substrate, as illustrated by means of a Lineweaver-Burk plot (Fig 8A). The  $K_m$  values for MS59 and WL64 were more than one order of magnitude lower than that for GOX: 19.5µM and 23.3µM for WL64 and MS59 respectively and 359µM for GOX. This means that the affinity for glucose is much higher for MS59 and WL64 than that of GOX. The  $V_{max}$  values were, however, comparable being 5.7, 16.8, and 9.7µmol H<sub>2</sub>O<sub>2</sub>/min/mg protein for WL64, MS59 and GOX, respectively.

Kinetic studies using the fungal cell wall suspension as substrate showed Michaelis-Menten kinetics for both MS59 and WL64, but

not for GOX as shown in Fig. 8B. The  $K_m$  values for MS59 and WL64 were 4.7 $\mu$ l and 24.3 $\mu$ l respectively, using the suspension described above. The  $V_{max}$  values were 22.0 and 11.2 $\mu$ mol  $H_2O_2$ /min/mg protein for respectively MS59 and WL64. Since GOX with fungal cell walls as  
 5 substrate does not show a linear relationship in a Lineweaver-Burk plot, the  $K_m$  and  $V_{max}$  could not be extrapolated from the plot. The kinetic data are summarized in table 4.

Table 4. Kinetic parameters of MS59, WL64 and glucose  
 10 oxidase

Enzyme	Glucose		Fungal Cell Wall Suspension	
	$K_m$ ( $\mu$ M)	$V_{max}$ ( $\mu$ mol $H_2O_2$ /min /mg)	$K_m$ ( $\mu$ l)	$V_{max}$ ( $\mu$ mol $H_2O_2$ /min/mg)
MS59	23.3	16.8	4.7	22.0
WL64	19.5	5.7	24.3	11.2
GOX	359	9.7		

#### EXAMPLE 10

##### Substrate specificities

Different substances were tested as possible substrates. Among  
 15 sucrose, sorbitol, fructose, c.m. cellulose,  $\beta$ -alanine, aspartic acid, chitine, cellulose, glutamate, glycine-glycine, laminarin, and glucose, only the latter served as substrate for at least WL64. Concentrations of the various substrates varied between 5mM and 50mM. It was further investigated whether glucose was the only substrate for  
 20 MS59 and WL64 or that other carbohydrates could also be oxidized. The enzyme assays were performed as described in Example 9, the substrate concentrations being 50mM. GOX was shown to oxidase glucose exclusively (Fig. 9). Same figure shows that MS59 and WL64 display a much broader substrate specificity, ranging from  $C_4$ -sugars to di- and  
 25 polysaccharides. The highest (and almost equal activities) were obtained with D-glucose, D-mannose, D-galactose, cellobiose, maltose, and lactose (Fig. 9). This range of substrates resembles the range found to be converted by hexose oxidase (EC. 1.1.3.5).

EXAMPLE 11

**Identification and characterization of genes homologous to  
the deduced MS59 nucleotide sequence**

Based on the amino acid sequences of pep1 (a.a. 12 to 22 of SEQ ID NO:  
5 1) and pep2 (a.a. 2 to 12 of SEQ ID NO: 2), primers were designated  
for PCR. Genomic DNA was isolated from sunflower cv. Zebulon and PCR  
primers 4 ( 5' AAC TTC TCC IAG IGT IGC ICC IGC TTG IAC CCA3', SEQ ID  
NO: 3) and 5 (5' GAT CCI TCT TTC CCI ATT ACT GGI GAG GTT TA3', SEQ ID  
NO: 4) were used to amplify a 354 bp DNA fragment from the sunflower  
10 genome with PCR. PCR products corresponding to this fragment size were  
cloned (SEQ ID NO: 5). Sequence analysis of the product revealed the  
presence of an uninterrupted Open Reading Frame (ORF) (SEQ ID NO: 6)  
of which the first and last stretch of amino acids corresponded with  
the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2. Several  
15 clones sequenced contained point mutations, varying from 1 to 4 in  
this PCR fragment. All but one of these mutations were silent  
mutations ( nucleotide nr 57 T to C, nucleotide nr 63 C to A,  
nucleotide nr 225 A to G) which therefore did not alter amino acid  
sequences encoded. One clone however did contain a point mutation (  
20 nucleotide nr 203 G to A) which altered the amino acid sequence at  
amino acid 68 from Arg to Lys.

A southern blot of sunflower genomic DNA, probed with SEQ ID NO: 5  
indicated the existence of multiple homologous sequences in the  
25 genome. Using SphI, 6 bands were detected, EcoRV 5 bands, SpeI 3 bands  
and NdeI 4 bands. With other enzymes 3-4 bands were previously  
discerned. This analysis suggests the existence of 3 genes with  
(partial) homology to the ms59 sequences.

30 New PCR primers were developed based on the non-variable areas between  
the original PCR primer sequences. Primers: for 3' RACE: 5' CAG GCA  
GCT GTG GTT TGT GGC 3' (SEQ ID NO: 7),  
for 5' RACE: 5' GTC CAC AAT GAA GAA GGG TTG 3' (SEQ ID NO: 8) and for  
nested 3' RACE: 5' ACG TAG ATA TCG AAC AAG AAA CCG C 3' (SEQ ID NO: 9).

35

Poly(A) containing RNA was isolated from sunflower leaf material that  
was induced by spraying 5 times with a 10 mM sodium salicylate  
solution. cDNA was prepared and 5' and 3' RACE PCR reactions were

performed as described in the instructions of the Marathon™ kit (Clontech laboratories, Inc., Palo Alto, CA). Partial cDNA clones were isolated by 5' and 3' RACE PCR reactions. Sequence analysis confirmed the identity of the partial cDNA clones.

- 5 Again new PCR and nested PCR primers were developed based on newly obtained sequence information from cloned 5' and 3' RACE PCR products. Primers for 5'RACE: 5' CTG GGG AAG CCC GTG TAG TAA AGC 3' (SEQ ID NO:11), 5' CGG GAA GTT GCA GAA GAT TGG GTT G 3' (SEQ ID NO:13), for nested 5'RACE: 5' GAG CAA GAG AAG AAG GAG AC 3' (SEQ ID NO:14), for 3' RACE: 5' GCT TTA CTA CAC GGG CTT CCC CAG 3' (SEQ ID NO: 10), and for nested 3' RACE: 5' GGT ACT CCA ACC ACG GCG CTC 3' (SEQ ID NO:12).
- 10 Four partial cDNA clones were isolated which together encode all of the Open Reading Frame including a putative signal peptide followed by an approximately 59 kDa protein, and 5' and 3' UTR's (untranslated regions)(SEQ ID NO: 15). A full length cDNA clone of 1784 bp, of which the ORF ( pos. 21 to pos. 1608) encodes 529 amino acid residues (SEQ ID NO:16), could be assembled out of these four partial cDNA clones and the PCR fragment mentioned above (SEQ ID NO: 5).
- 15 The amino-terminal signal sequence (Von Heijne et al., 1983 and Von Heijne, 1985) is not likely fully presented within the first 19 amino acid residues. A prediction of the putative cleavage site was made.
- 20

- The amino acid sequence of this cDNA clone was used in a BLAST homology search. This sequence revealed high homology to the Berberine Bridge Enzymes (BBE) from Californian poppy (*Eschscholtzia californica*) (Dittrich and Kutchan, 1991, Proc. Natl. Acad. Sci. USA 88, 9969-9973) and Opium poppy (*Papaver somniferum*) (Facchini et al., 1996, Plant Physiol. 112, 1669-1677).
- 25

- BLAST screening of Expressed Sequence Tag (dbEST) databases with the amino acid sequence as shown in SEQ ID NO: 16 revealed homologues of the MS59 protein in *Arabidopsis thaliana* (SEQ ID NO: 21 to SEQ ID NO: 47) and rice (SEQ ID NO: 48).
- 30

- The EST sequences are listed in the sense orientation considering the orientation of homology to MS59. Sequences of the EST clones were altered by inserting one or two extra unknown nucleotides (N or NN) at frameshift positions in order to obtain one single translation frame with homology to MS59.
- 35



**EXAMPLE 12****Isolation of the gene encoding WL64 and determination of the nucleotide sequence**

Based on the amino acid sequence of the amino-terminus of the  
5 WL64 protein ( SEQ ID NO: 49, Thr-Ser-Thr-Ser-Ile-Ile-Asp-Arg-Phe-Thr-  
Gln-(Cys/Ser)-Leu-Asn-Asn-Arg-Ala-Asp-Pro-(Ser)-(Phe)-) a primer (a.a.  
1 to 11 of SEQ ID NO: 49) was developed for PCR.  
The N-terminal amino acid sequence (SEQ ID NO: 49) revealed high  
homology to the corresponding portion of the MS59 protein (amino acid  
10 residues 20 to 39 in SEQ ID NO: 16).  
cDNA was prepared from Poly(A) containing RNA that was isolated from  
lettuce (*Lactuca sativa* cv. Lollo bionda) leaves that were induced by  
spraying 5 times with a 10 mM sodium salicylate solution. PCR primers  
FR-WL64-142 ( 5'ACT TCT ACT TCT ATT ATT GAT AGG TTT ACT CA3', SEQ ID  
15 NO: 52) and MS59 primer 4 (5'AAC TTC TCC IAG IGT IGC ICC IGC TTG IAC  
CCA3', SEQ ID NO: 3) were used to amplify a 405 bp fragment from the  
lettuce cDNA pool. PCR products corresponding to this PCR fragment  
were cloned and sequenced (SEQ ID NO: 53) and revealed an  
uninterrupted open reading frame (SEQ ID NO: 54).

**Table 5. EST sequences showing homology to MS59.**

Frameshifts were introduced for optimal aligning of the EST's with the MS59 sequence. In the columns with frameshift 1 and frameshift 2 the position of the frameshift and the shift (frame--->frame) are listed.

5 The (-) mark means, no frameshift present.

SEQ ID NO:	EST name	GenBank acces- sion	Frame (1, 2, 3)	Frameshift 1	Frameshift 2
21	ATTS5925	F19886	2	-	-
22	ATTS0345	Z17771	2	202, 2 ---> 1	-
23	ATTS5268	F14356	2	298, 2 ---> 3	-
24	TC13883	-	1	177, 1 ---> 2	-
25	TC11550	-	2	-	-
26	P_16053	R84094	1	-	-
27	P_22214	N97049	2	310, 2 ---> 3	-
28	P_16873	R90518	3	317, 3 ---> 2	-
29	ATTS2532	Z30784	3	188, 3 ---> 1	312, 1 ---> 2
30	TC11456	-	3	-	-
31	P_8818	T45555	1	98, 1 ---> 3	-
32	P_21340	N96011	1	-	-
33	P_22585	W43206	2	367, 2 ---> 1	-
34	Q_ATTS2533	Z30785	2	-	-
35	P_17333	H76902	2	-	-
36	P_9615	T46352	1	-	-
37	Q_ATTS2959	Z33920	2	-	-
38	P_2730	T20722	1	-	-
39	TC9870	-	2	-	-
40	P_14876	H36354	2	241, 2 ---> 1	-
41	P_21353	N96040	1	89, 1 ---> 2	-
42, 43	Q_ATTS3343	Z34583	1	-	-
44	Q_ATTS4954	F14032	2	139, 2 ---> 1	-
45	Q_ATTS1606	Z26512	2	-	-
46	P_7866	T44603	1	222, 1 ---> 3	-
47	AA0410042	24308	2	421, 2 ---> 1	-
48	RICS2381A	D40415	3	-	-

New PCR primers were developed based on the sequence of SEQ ID NO: 53 that is located between the original PCR primers. Primers for 5' RACE: 5'CAC GTT TAT GGA GCG TAA GTT GAA C3' (SEQ ID NO: 55) and for 3' RACE: 5'CAC CCT TCA CAC ATT CAA GCA GC3' (SEQ ID NO: 56) were synthesized and used in 5' and 3' RACE PCR reactions, performed as described in the instructions of the Marathon™ cDNA amplification kit (Clontech laboratories, Inc., Palo Alto, CA). Two partial cDNA clones were amplified by 5' and 3' RACE reactions. Sequence analysis confirmed the identity of the partial cDNA clones which together encode all of the open reading frame including a putative signal peptide and 5' and 3' UTR's (untranslated regions). A full length cDNA clone of 1981 bp (SEQ ID NO: 57) was assembled of which the ORF (pos. 7 to pos. 1629) encodes 540 amino acid residues (SEQ ID NO: 58). The amino terminal signal sequence is represented by the first 27 amino acid residues.

15

### EXAMPLE 13

#### **Characterization and isolation of Berberine Bridge Enzyme genes from *Papaver somniferum* and *Eschscholtzia californica***

Genomic DNA was prepared from leaves of full grown Californian poppy (*Eschscholtzia californica*) and Opium poppy (*Papaver somniferum* cv Marianne) plants.

Primers were designed for the Californian poppy gene (EcBBE) at the start of the mature protein (5' GGT AAT GAT CTC CTT TCT TGT TTG ACC 3', SEQ ID NO: 59) and at the stop codon introducing a Not I restriction site just downstream of the TAG stop codon (5' AGA GCG GCC GCT ATA TTA CAA CTT CTC CAC CAT CAC TCC TC 3', SEQ ID NO: 60).

For the Opium poppy gene (PsBBE) primers were designed in a similar way at the start of the presumed mature protein (5' GGT GAT GTT AAT GAT AAT CTC CTC 3', SEQ ID NO: 61) and at the TAG stop codon introducing a Not I restriction site (5' AGA GCG GCC GCT ACA ATT CCT TCA ACA TGT AAA TTT CCT C 3', SEQ ID NO: 62).

These primers were used to amplify the mature portion of both the BBE genes.

The PCR products were digested with Not I and ligated into vector pET32a (Novagen, Madison, WI) digested with EcoR V and Not I. The correct insertion of the fragment was confirmed using restriction enzyme analysis and DNA sequencing.

EXAMPLE 14

**Characterization and isolation of MS59 homologues from  
*Arabidopsis thaliana***

- In our blast screening we identified 26 EST's with homology to MS59. One EST was found in Rice and the remaining 25 were all found in *A. thaliana*. Homologous EST's were found over the entire length of the MS59 sequence. Analysis of the *Arabidopsis* expressed sequence tags revealed that there are 3 EST's with high homology at the 5' end of the protein (SEQ ID NO: 21, SEQ ID NO: 39 and SEQ ID NO: 40) of which SEQ ID NO: 39 and SEQ ID NO: 40 are overlapping sequences. The 3' part of MS59 showed homology to 7 EST sequences (SEQ ID NO: 24, 27, 32, 34, 41, 43 and 45) of which SEQ ID NO: 24 is overlapping with SEQ ID NO: 43 and SEQ ID NO: 32 is overlapping with SEQ ID NO: 45.
- Primers were designed, located at the start of the presumed mature part (possible cleavage sites were predicted according to consensus sequences described by Von Heijne et al., 1983 and Von Heijne, 1985) of the two different EST's homologous with the 5' part of MS59 (SEQ ID NO: 16).
- The EST sequence represented by SEQ ID NO: 21 possibly missed the first three amino acid residues of the predicted mature part when compared to the MS59 amino acid sequence (SEQ ID NO: 16) and, therefore, A.a. residues 20 to 22 of SEQ ID NO: 16 were introduced by including 9 nucleotides at the 5' end of the primer.
- Primer located 5' in SEQ ID NO: 21, adding residues 20 to 22 of MS59 (SEQ ID NO: 16): 5' ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC 3' (SEQ ID NO: 63).
- Primer located behind the predicted cleavage site of SEQ ID NO: 39 and SEQ ID NO: 40: 5' TCC ATC CAA GAT CAA TTC ATA AAC TGT GTC (SEQ ID NO: 64).

Primers were also made located around the stopcodon of the five different EST's homologous with the 3' part of the MS59 a.a. sequence (SEQ ID NO: 16) and introducing a Not I restriction site for cloning in the pET32a *E. coli* expression vector.

Primer located in SEQ ID NO: 24 and SEQ ID NO: 43, 5' AGA GCG GCC GCT TTC ATG AAC CTA GCT TCT AGT AGG 3' (SEQ ID NO: 65). Primer in SEQ ID NO 27, 5' AGA GCG GCC GCG AAA TGG CCC CCC TTT TAA AAC GGG G 3' (SEQ ID

NO: 66). Primer in SEQ ID NO:32 and SEQ ID NO: 41, 5' AGA GCG GCC GCA  
AAT GAT ATC TTC AGG TAA CTT TGT TCA C (SEQ ID NO: 67). Primer in SEQ  
ID NO: 34, 5' AGA GCG GCC GCA TAA TCA AAT AAA TAC ACT TAT GGT AAC ACA  
G (SEQ ID NO: 68) and the primer in SEQ ID NO: 45, 5' AGA GCG GCC GCT  
5 GGT TTT GTA TTG AGG ACT CAA AAC AG 3' (SEQ ID NO: 69).

All possible combinations of the 5' primers with the 3' primers were  
used in a PCR on genomic DNA isolated from *Arabidopsis thaliana* cv  
Columbia. In a PCR with the primers SEQ ID NO: 63 and SEQ ID NO: 68 an  
10 approximately 1800 bp band was amplified. This band was cloned and  
identity of the PCR product was confirmed by DNA sequencing. The  
cloned PCR product of 1757 bp (SEQ ID NO: 70) contained an intron from  
position 570 to position 801, the open reading frame of SEQ ID NO: 70  
consists of 508 amino acid residues (SEQ ID NO: 71).

15 Total RNA was isolated from *Arabidopsis thaliana* Col-0 from 12 days  
old sterile etiolated seedlings grown in the dark on Murashige and  
Skoog agar, from 12 days old sterile seedlings grown in liquid  
Murashige and Skoog medium with a 16 hour photoperiod and from leaves,  
stems, flowers and siliques from full grown plants (Newman et al.,  
20 1994 Plant Physiol. 106: 1241-1255). The RNA from the different  
developmental stages was pooled. Poly(A)<sup>+</sup> RNA was isolated using the  
Poly(A) Quick® mRNA Isolation kit (Stratagene, La Jolla, CA) and cDNA  
was prepared using the Marathon™ cDNA Amplification Kit (Clontech  
Laboratories Inc., Palo Alto, CA).

25

PCR reactions were set up with the cDNA pool with different  
combinations of 5' primers and 3' primers. A PCR product was amplified  
with the primer combination SEQ ID NO: 63 and SEQ ID NO: 68 of  
approximately 1600 bp. The PCR product was cloned in the *EcoR* V and  
30 *Not* I restriction sites of the bacterial expression vector pET32a  
(Novagen, Madison, WI). The sequence of the PCR product was determined  
and revealed an uninterrupted open reading frame of 1527 bp (SEQ ID  
NO: 72) representing a protein of 508 amino acid residues (SEQ ID NO:  
73).

35

A second cDNA clone of about 1600 bp was amplified with the primer  
combination SEQ ID NO: 64 and SEQ ID NO: 65. This cDNA clone was also  
ligated into the *EcoR* V and *Not* I restriction sites of pET32a

(Novagen, Madison, WI). This cDNA PCR clone was also characterized by DNA sequencing and consisted of an uninterrupted open reading frame of 1530 bp (SEQ ID NO: 74) encoding 509 amino acid residues (SEQ ID NO: 75).

5

#### EXAMPLE 15

**Expression of MS59, the Berberine Bridge Enzymes from *Papaver somniferum* and *Eschscholtzia californica* and two homologous proteins from *Arabidopsis thaliana* cv Columbia in *E. coli***

10

A PCR fragment containing the presumed mature portion of MS59 was introduced in vector pET32c (Novagen, Madison, WI), and the correct insertion of the fragment is confirmed using DNA sequencing. Then, the plasmid was introduced into *E. coli* AD494 (DE3) pLySS (Novagen, Madison, WI). Small scale cultures (2 ml) of several colonies were then started of which half is induced by the addition of IPTG to 1mM final concentration. Total extracts from *E. coli* were run on SDS gels and analyzed by Coomassie Brilliant Blue staining. Several clones exhibited strong overexpression of the MS59 protein. A clone which had strong overexpression was selected for a large scale culture. Five hundred ml of LB supplemented with 0.4 mM glucose was inoculated with a culture of this *E. coli* and grown to an optical density of 0.5-0.7. Then, IPTG was added to a final concentration of 1 mM and protein production allowed for 3 hours at 30°C. A large proportion of the MS59 protein was found in the insoluble protein fraction, a small amount appeared soluble. The resulting insoluble protein preparation contained mainly MS59 protein. This preparation is used for raising antibodies (Example 17). The soluble fraction was used in an *in vitro* assay to test whether the MS59 protein still exhibited antifungal activity.

15

20

25

30

The pET32a plasmids containing the open reading frames of the four MS59/WL64 homologues were introduced into *E. coli* AD494(DE3)pLySS (Novagen, Madison, WI). Small scale cultures (25 ml) of several independent clones were grown to an optical density of 0.5-0.7. Then IPTG was added to a final concentration of 1mM and protein production was allowed for 4 hours at 30°C.

35

Soluble and total protein fractions were isolated. The samples were analyzed using SDS-PAGE followed by Neuhoff staining and Western

analysis using the S-Tag Western Blotting detection kit (Novagen, Madison, WI). A large portion of the protein was found in the insoluble fraction, only a small amount appeared to be soluble. Clones which strongly overexpressed the homologous proteins were selected for production of the proteins in large scale cultures of 1.5 liter each.

#### EXAMPLE 16

In vitro antifungal assays of MS59, MS59/WL64 homologues from Californian poppy (*Eschscholtzia californica*) and Opium poppy (*Papaver somniferum*) and two homologous proteins from *Arabidopsis thaliana*

The MS59 protein produced in *E. coli* contained N-terminal trxA-, His- and S-Tags. The His-tag was used for purification of the soluble MS59 on an IMAC (immobilized metal affinity chromatography) column, charged with  $\text{Ni}^{2+}$ . Bound protein was eluted by increasing the imidazole concentration. The peak fraction from this purification contains some contaminating *E. coli* proteins.

The peak fraction of this MS59 purification was dialysed into 50 mM MES, pH 6.0, and used in an in vitro assay with *Phytophthora infestans* and *Pythium ultimum*. For the standard setup of the in vitro antifungal assay with *Phytophthora infestans* and *Pythium ultimum* see above.

As control treatment we assayed an unrelated His-tagged protein purified from the same expression host, with some *E. coli* protein background. Also a boiled MS59 control (heated 10 minutes at 100°C) was included. Approximately 40 ng of fusion protein was tested in the *Phytophthora infestans* assay, twice that amount was used for the *Pythium ultimum* inhibition assay.

Microtiter dishes were wrapped with Parafilm and incubated in the dark at room temperature. After 2-3 days the mycelium of the growing fungus in the wells was stained with lactophenol cotton blue and the extent of growth was estimated.

IMAC fractions from the soluble fraction of *E. coli* containing MS59 showed complete inhibition of *P. infestans* and *P. ultimum* at concentrations of 20-40 ng.

Table 6. Antifungal effects of MS59 from *E.coli* on *Phytophthora infestans*

Fraction	MS59 <i>E. coli</i>	MS59 <i>E. coli</i> boiled	His- protein <i>E.coli</i>	MES buffer
Growth inhibition	4	0	0	0
amount of extract	5µl	5µl	5µl	

5 Growth inhibition (GI) is scored visually on a linear scale of 0 (no inhibition) to 4 (complete growth inhibition).

Table 7. Antifungal effects of MS59 from *E.coli* on *Pythium ultimum*

Fraction	MS59 <i>E. coli</i>	MS59 <i>E. coli</i> boiled	His- protein <i>E.coli</i>	MES buffer
Growth inhibition	4	0	0	0
amount of extract	10µl	10µl	10µl	

10

Microscopical analysis of the wells indicate the rapid germination and subsequent growth of *Phytophthora infestans* zoospores in each of the controls. Germination is near completely inhibited in the reactions containing the MS59 protein from *E. coli*. Some spores do germinate, but hyphal tip growth appears to stop soon after initiation. After 48 hours growth of *Phytophthora infestans* mycelium is abundant in the controls, but almost undetectable in the assay containing MS59. Even after 72 hours no substantial growth is observed. Fungal hyphae appear somewhat granular and thickened in the reactions containing MS59 protein. Examples of the characteristic patterns of fungal growth in incubations with and without *E. coli*-



produced MS59 are depicted in figure 4. After 48 and 72 hours fungal growth in the control incubations is so extensive no photographic material could be gathered. Incubations in the presence of MS59 leads to complete blockage of further growth, the germination tubes observed at 24 hours do not noticeably extend further.

Likewise, in the *Pythium ultimum* inhibition assay, where mycelium fragments are used, no growth is apparent upon treatment with MS59 (see fig. 5). After 24 hours the control reactions were completely overgrown by mycelium. Only small mycelium fragments are at that stage apparent in the MS59-treated sample.

The poppy homologues were expressed in *E. coli* (pET32a) and tested for *in vitro* antifungal activity on *Phytophthora infestans* and *Pythium ultimum*. *Phytophthora infestans* spores and hyphal fragments of *Pythium ultimum* were suspended in respectively sterile water or potato dextrose broth (PDB). 400-600 spores or 200 fragments/50 µl were added to each well.

The expressed proteins were partially purified by means of IMAC column chromatography. Fractions containing the expressed proteins were buffer exchanged to 50mM MES, pH 6.0, filter sterilized, and tested for their antifungal activity, with IMAC purified *E. coli* pET32-MS59 as a positive control.

No antifungal activity was observed for both *Eschscholtzia californica* and *Papaver somniferum* MS59/WL64 homologues, not even at concentrations ten times higher than that of the positive control, leading to a 100% growth inhibition of both fungi. This could mean that these *E. coli* expressed proteins did not have the correct folding and therefore showed no biological activity.

#### EXAMPLE 17

##### **Raising antibodies against denatured MS59**

Antibodies were raised in rabbits against denatured MS59 (the solubilized form from the insoluble *E. coli* fraction) in PAG-slices. The antibodies showed cross-reaction with a band of about 60 kDa only in the IF of pMOG1180 (Examples 18 and 19) tobacco plants containing antifungal and glucose oxidase activity. Surprisingly, no cross-reaction with WL64 is found.

EXAMPLE 18**Tailoring a MS59 clone for expression in transgenic plants**

- PCR primers were developed based on the sequence around the ATG start codon and the TGA stop codon for cloning of the open reading frame (ORF). A NcoI restriction site was introduced at the ATG start codon for fusion to a constitutive promoter by PCR using primer: 5' CC GCC ATG GAG ACT TCC ATT CTT ACT C 3' (SEQ ID NO:16). The second codon of the ORF was changed from caa (Q) to gag (E) as a result of the introduced NcoI restriction site.
- Downstream of the TGA stop codon a BamHI restriction site was introduced by PCR using primer: 5' GCC GGA TCC TCA AGA TGA CAA AGT TGG GAT GCT 3' (SEQ ID NO:18).
- Using a PCR reaction with *Pfu* DNA polymerase, we amplified the entire ORF, using the PCR primers to introduce the NcoI restriction site on the startcodon ATG and the BamHI restriction enzyme recognition site just downstream of the stopcodon. The integrity of the DNA sequence was confirmed by sequencing (SEQ ID NO:19). The entire ORF was linked to a constitutive promoter which allows high level protein expression in most parts of the plant. After the ORF a 3' untranslated region of the potato proteinase inhibitor II (Thornburg et al., 1987, Proc. Natl. Acad. Sci. USA 84, 744-748), which contains sequences needed for polyadenylation (An et al., 1989, Plant Cell 1, 115-122), was introduced. The chimeric gene produced was introduced into binary vector pMOG800 (deposited at the Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands, under CBS 414.93, on August 12, 1993). The resulting clone pMOG1180, which harbours the MS59 construct under control of the ocs-mas hybrid promoter (WO95/14098) was introduced in *Agrobacterium tumefaciens* strain EHA105, suitable for transformation of target crops tomato and potato, strain MOG101 for transformation of tobacco and *Arabidopsis* and MOG301 for transformation of *Brassica napus*.

**EXAMPLE 19****Production and analysis of transgenic tobacco and potato plants containing the MS59 gene construct**

Using *Agrobacterium* mediated transformation system binary

5 constructs containing the MS59 gene construct as described in Example 18 were introduced into tobacco and potato. The transgenic shoots of these different plant species were regenerated into whole plants and subsequently, primary transformants were analyzed for expression of the newly introduced MS59 gene. For this analysis use was made of  
10 Western blotting techniques, using antibodies against MS59 specific peptide coupled to BSA. All antisera were diluted 1:5,000. A concentration series of purified proteins (12.5, 25, 50 and 100 ng) was used to judge the expression level of the introduced proteins in the transgenic plants. Transgenic samples were homogenized in 50 mM  
15 sodium acetate buffer pH 5.2 and the extracts were clarified by centrifugation. The supernatants were either directly analyzed or left overnight to precipitate on ice. Overnight precipitation was always followed by clarification (by centrifugation). The protein concentration of the supernatants obtained in either way was  
20 determined using Bradford reagent (Bradford 1976, Anal. Biochem. 72, 248-254) and BSA as the standard protein. As much protein as possible (but never more than 10 µg) was loaded on a 12,5 % SDS-PAA gel (Laemmli, supra) and immunoblotted as previously described (Ponstein et al. supra).

25

Extracts from leaves of ms59-transgenic tobacco and potato plants were made by pottering leaf fragments in a buffer containing 50 mM NaAc, (pH = 5.2). After this, insoluble protein was removed by centrifugation. Total soluble protein content was measured and the  
30 equivalent of 10 µg was loaded on a SDS-gel. After running the gel the proteins were transferred to blot. This blot was developed using the antiserum raised against purified MS59 (Example 17). The MS59-specific antiserum was used in a 1:5,000 dilution. Purified MS59 was also run alongside on the gel, and is included for reference.  
35 A number of transformed plants selected based on their high level expression of MS59 protein and S1 progeny plants will be tested in fungal infection assays.

**EXAMPLE 20****Purification of MS59 transproteins from tobacco transgenics**

Transgenic tobacco plants were produced expressing MS59 constitutively. Levels of expression are determined using Western analysis. Extracts of the transgenic material are assayed for *in vitro* growth inhibitory activity against *Phytophthora infestans* and *Pythium ultimum*. Small scale total extracts were made from *in vitro* leaves of tobacco containing the pMOG1180 construct (mas-ocs-promotor-MS59) and of tobacco control lines. The extracts were made by grinding leaf material in 50 mM NaAc pH 5.2. The supernatant was dialysed against 50 mM MES pH 6.0 and tested for *in vitro* antifungal activity according to the methods described in the general experimental part. Some of the tobacco pMOG1180 lines showed high antifungal activity on *P. infestans* and *P. ultimum* compared to other lines or control lines.

**EXAMPLE 21****Carbohydrate oxidase activity / Localization of MS59 in transgenic tobacco**

Equal amounts of partial purified soluble MS59 and soluble homologue fractions (Papaver, Eschscholzia, Arabidopsis-A11 and -B7) were tested for carbohydrate oxidase activity. Carbohydrate oxidase activity for MS59 was 0.011 ODu/min and for the homologues 0.0003-0.0012 ODu/min, a difference of a factor 10.

From the transgenic pMOG1180 tobacco lines of Example 20 that showed antifungal activity *in vitro* IF was isolated at a later stage and tested for carbohydrate oxidase activity. Also the material that was left after IF isolation (called "-IF ") was tested. The same lines that showed antifungal activity have high carbohydrate oxidase activity. The activity is located in the IF.

EXAMPLE 22

Introduction of the four genes construct containing Chi-I, Glu-I, AP24 and MS59 under control of a constitutive plant promoter, into tomato, potato, carrot, *Brassica napus* and

5

*Arabidopsis*

Using *Agrobacterium* mediated transformation system binary construct pMOG1145 and pMOG1180 containing the genes encoding Chi-I, Glu-I, AP24 and MS59 or pMOG1146 containing the genes encoding Chi-I, Glu-I, bPR-1 and MS59 is introduced into different crop species  
10 including, tomato, potato, carrot, *Brassica napus* and *Arabidopsis*.  
S1 progeny plants are tested in fungal infection assays.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT:  
(A) NAME: MOGEN International nv  
(B) STREET: Einsteinweg 97  
(C) CITY: Leiden  
(E) COUNTRY: The Netherlands  
10 (F) POSTAL CODE (ZIP): 2333 CB  
(G) TELEPHONE: 31-(0)71-5258282  
(H) TELEFAX: 31-(0)71-5221471
- (ii) TITLE OF INVENTION: Antifungal proteins, DNA coding therefor,  
15 and hosts incorporating same.
- (iii) NUMBER OF SEQUENCES: 75
- (iv) COMPUTER READABLE FORM:  
20 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)
- 25 (vi) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: EP 96.202.466.7  
(B) FILING DATE: 04-SEP-1996
- (vi) PRIOR APPLICATION DATA:  
30 (A) APPLICATION NUMBER: EP 97.200.831.2  
(B) FILING DATE: 10-MAR-1997

## (2) INFORMATION FOR SEQ ID NO: 1:

- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- 45 (iii) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:  
50 (A) ORGANISM: Helianthus annuus  
(B) STRAIN: cv. zebulon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly  
 1 5 10 15  
 Ala Thr Leu Gly Glu Val Tyr Tyr Arg  
 20 25

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Helianthus annuus*

(B) STRAIN: cv. zebulon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr Pro Gly Xaa Ser  
 1 5 10 15  
 Ser Phe Pro Thr Val Leu Gln Asn Tyr  
 20 25

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AACTTCTCCN AGNGTNGCNC CNGCTTGNAC CCA

33

5 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: YES

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1

20 (D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

25 GATCCNTCTT TCCCNATTAC TGGNGAGGTT TA

32

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 354 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

40

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Helianthus annuus

(B) STRAIN: cv. zebulon

45 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..354

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GAT CCG TCT TTC CCG ATT ACT GGG GAG GTT TAC ACT CCC GGA AAC TCA  
Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr Pro Gly Asn Ser

48

1

5

10

15

55



TCT TTT CCT ACC GTC TTG CAA AAC TAC ATC CGA AAC CTT CGG TTC AAT 96  
 Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn  
 20 25 30

5 GAA ACT ACC ACA CCA AAA CCC TTT TTA ATC ATC ACA GCC GAA CAT GTT 144  
 Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr Ala Glu His Val  
 35 40 45

10 TCC CAC ATT CAG GCA GCT GTG GTT TGT GGC AAA CAA AAC CGG TTG CTA 192  
 Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln Asn Arg Leu Leu  
 50 55 60

15 CTG AAA ACC AGA AGC GGT GGT CAT GAT TAT GAA GGT CTT TCC TAC CTT 240  
 Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu  
 65 70 75 80

ACA AAC ACA AAC CAA CCC TTC TTC ATT GTG GAC ATG TTC AAT TTA AGG 288  
 Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met Phe Asn Leu Arg  
 85 90 95

20 TCC ATA AAC GTA GAT ATC GAA CAA GAA ACC GCA TGG GTC CAA GCC GGC 336  
 Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly  
 100 105 110

25 GCC ACC CTC GGA GAA GTT 354  
 Ala Thr Leu Gly Glu Val  
 115

30 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 amino acids

(B) TYPE: amino acid

35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

40 Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr Pro Gly Asn Ser  
 1 5 10 15

Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn  
 45 20 25 30

Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr Ala Glu His Val  
 35 40 45

50 Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln Asn Arg Leu Leu  
 50 55 60

Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu  
 65 70 75 80

55

Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met Phe Asn Leu Arg  
                             85                            90                            95

5 Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly  
                             100                            105                            110

Ala Thr Leu Gly Glu Val  
                             115

10 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- 25 (A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1  
 (D) OTHER INFORMATION: /function= "primer"

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CAGGCAGCTG TGGTTTGTGG C

21

(2) INFORMATION FOR SEQ ID NO: 8:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- 50 (A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1  
 (D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

55 GTCCACAATG AAGAAGGGTT G

21

## (2) INFORMATION FOR SEQ ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /function= "primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACGTAGATAT CGAACAAGAA ACCGC

25

## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

40 GCTTTACTAC ACGGGCTTCC CCAG

24

## (2) INFORMATION FOR SEQ ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTGGGGAAGC CCGTGTAGTA AAGC

24

5 (2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20

GGTACTCCAA CCACGGCGCT C

21

(2) INFORMATION FOR SEQ ID NO: 13:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35 (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CGGGAAGTTG CAGAAGATTG GGTG

25

40

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

45 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GAGCAAGAGA AGAAGGAGAC

20

5 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1784 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

15 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

20 (A) ORGANISM: *Helianthus annuus*

(B) STRAIN: Zebulon

(ix) FEATURE:

(A) NAME/KEY: CDS

25 (B) LOCATION: 21..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

30	ATATCACATC TTCTTTCAAC ATG CAA ACT TCC ATT CTT ACT CTC CTT CTT	50
	Met Gln Thr Ser Ile Leu Thr Leu Leu Leu	
	1 5 10	
35	CTC TTG CTC TCA ACC CAA TCT TCT GCA ACT TCC CGT TCC ATT ACA GAT	98
	Leu Leu Leu Ser Thr Gln Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp	
	15 20 25	
40	CGC TTC ATT CAA TGT TTA CAC GAC CGG GCC GAC CCT TCA TTT CCG ATA	146
	Arg Phe Ile Gln Cys Leu His Asp Arg Ala Asp Pro Ser Phe Pro Ile	
	30 35 40	
	ACC GGA GAG GTT TAC ACT CCC GGA AAC TCA TCT TTT CCT ACC GTC TTG	194
	Thr Gly Glu Val Tyr Thr Pro Gly Asn Ser Ser Phe Pro Thr Val Leu	
	45 50 55	
45	CAA AAC TAC ATC CGA AAC CTT CGG TTC AAT GAA ACT ACC ACA CCA AAA	242
	Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys	
	60 65 70	
50	CCC TTT TTA ATC ATC ACA GCC GAA CAT GTT TCC CAC ATT CAG GCA GCT	290
	Pro Phe Leu Ile Ile Thr Ala Glu His Val Ser His Ile Gln Ala Ala	
	75 80 85 90	
55	GTG GTT TGT GGC AAA CAA AAC CGG TTG CTA CTG AAA ACC AGA AGC GGT	338
	Val Val Cys Gly Lys Gln Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly	
	95 100 105	

	GGT CAT GAT TAT GAA GGT CTT TCC TAC CTT ACA AAC ACA AAC CAA CCC	386
	Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro	
	110 115 120	
5	TTC TTC ATT GTG GAC ATG TTC AAT TTA AGG TCC ATA AAC GTA GAT ATC	434
	Phe Phe Ile Val Asp Met Phe Asn Leu Arg Ser Ile Asn Val Asp Ile	
	125 130 135	
	GAA CAA GAA ACC GCA TGG GTC CAA GCC GGT GCG ACT CTT GGT GAA GTG	482
10	Glu Gln Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu Gly Glu Val	
	140 145 150	
	TAC TAT CGA ATA GCG GAG AAA AGT AAC AAG CAT GGT TTT CCG GCA GGG	530
	Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Lys His Gly Phe Pro Ala Gly	
15	155 160 165 170	
	GTT TGT CCA ACG GTT GGC GTT GGT GGG CAT TTT AGT GGT GGT GGG TAT	578
	Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly Gly Gly Tyr	
	175 180 185	
20	GGT AAT TTG ATG AGA AAA TAT GGT TTG TCG GTT GAT AAT ATT GTT GAT	626
	Gly Asn Leu Met Arg Lys Tyr Gly Leu Ser Val Asp Asn Ile Val Asp	
	190 195 200	
25	GCT CAA ATA ATA GAT GTG AAT GGC AAG CTT TTG GAT CGA AAG AGT ATG	674
	Ala Gln Ile Ile Asp Val Asn Gly Lys Leu Leu Asp Arg Lys Ser Met	
	205 210 215	
	GGT GAG GAT TTG TTT TGG GCG ATC ACC GGC GGT GGT GGT GTT AGT TTT	722
30	Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly Val Ser Phe	
	220 225 230	
	GGT GTG GTT CTA GCC TAC AAA ATC AAA CTA GTT CGT GTT CCG GAG GTT	770
	Gly Val Val Leu Ala Tyr Lys Ile Lys Leu Val Arg Val Pro Glu Val	
35	235 240 245 250	
	GTG ACC GTG TTT ACC ATT GAA AGA AGA GAG GAA CAA AAC CTC AGC ACC	818
	Val Thr Val Phe Thr Ile Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr	
	255 260 265	
40	ATC GCG GAA CGA TGG GTA CAA GTT GCT GAT AAG CTA GAT AGA GAT CTT	866
	Ile Ala Glu Arg Trp Val Gln Val Ala Asp Lys Leu Asp Arg Asp Leu	
	270 275 280	
45	TTC CTT CGA ATG ACC TTT AGT GTC ATA AAC GAT ACC AAC GGT GGA AAG	914
	Phe Leu Arg Met Thr Phe Ser Val Ile Asn Asp Thr Asn Gly Gly Lys	
	285 290 295	
	ACA GTC CGT GCT ATC TTT CCA ACG TTG TAC CTT GGA AAC TCG AGG AAT	962
50	Thr Val Arg Ala Ile Phe Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn	
	300 305 310	
	CTT GTT ACA CTT TTG AAT AAA GAT TTC CCC GAG TTA GGG TTG CAA GAA	1010
	Leu Val Thr Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu	
55	315 320 325 330	

TCG GAT TGT ACT GAA ATG AGT TGG GTT GAG TCT GTG CTT TAC TAC ACG 1058  
 Ser Asp Cys Thr Glu Met Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr  
 335 340 345

5 GGC TTC CCC AGT GGT ACT CCA ACC ACG GCG CTC TTA AGC CGT ACT CCT 1106  
 Gly Phe Pro Ser Gly Thr Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro  
 350 355 360

10 CAA AGA CTC AAC CCA TTC AAG ATC AAA TCC GAT TAT GTG CAA AAT CCT 1154  
 Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro  
 365 370 375

15 ATT TCT AAA CGA CAG TTC GAG TTC ATC TTC GAA AGG CTG AAA GAA CTT 1202  
 Ile Ser Lys Arg Gln Phe Glu Phe Ile Phe Glu Arg Leu Lys Glu Leu  
 380 385 390

GAA AAC CAA ATG TTG GCT TTC AAC CCA TAT GGT GGT AGA ATG AGT GAA 1250  
 Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu  
 395 400 405 410

20 ATA TCC GAA TTC GCA AAG CCT TTC CCA CAT AGA TCG GGT AAC ATA GCG 1298  
 Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly Asn Ile Ala  
 415 420 425

25 AAA ATT CAA TAC GAA GTA AAC TGG GAG GAT CTT AGC GAT GAA GCC GAA 1346  
 Lys Ile Gln Tyr Glu Val Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu  
 430 435 440

30 AAT CGT TAC TTG AAT TTC ACA AGG CTG ATG TAT GAT TAC ATG ACC CCA 1394  
 Asn Arg Tyr Leu Asn Phe Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro  
 445 450 455

35 TTT GTG TCG AAA AAC CCT AGA AAA GCA TTT TTG AAC TAT AGG GAT TTG 1442  
 Phe Val Ser Lys Asn Pro Arg Lys Ala Phe Leu Asn Tyr Arg Asp Leu  
 460 465 470

GAT ATT GGT ATC AAC AGC CAT GGC AGG AAT GCT TAT ACT GAA GGA ATG 1490  
 Asp Ile Gly Ile Asn Ser His Gly Arg Asn Ala Tyr Thr Glu Gly Met  
 475 480 485 490

40 GTT TAT GGG CAC AAG TAT TTC AAA GAG ACA AAT TAC AAG AGG CTA GTA 1538  
 Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val  
 495 500 505

45 AGT GTG AAG ACT AAA GTT GAT CCT GAC AAC TTC TTT AGG AAT GAG CAA 1586  
 Ser Val Lys Thr Lys Val Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln  
 510 515 520

50 AGC ATC CCA ACT TTG TCA TCT T GAAGAACGTA CATATATAAA TAAATACCTT 1638  
 Ser Ile Pro Thr Leu Ser Ser  
 525

TGTGCATGGT ATTTTCAGGG TGTTAAAGTG ATATTCAGAT ATTTATGATA GAATTTTGAC 1698

55 TTGTATTTTA TACAATCAAA ATTGATGGT TCTCCGAATT TCTCTTTTAA ATTCTGAAAA 1758

ATACATATTA GTATTGTCAA AAAAAA

1784

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 529 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

15 Met Gln Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Ser Thr Gln  
     1                    5                    10                    15  
  
 Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu  
                     20                    25                    30  
 20 His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr  
                     35                    40                    45  
  
 Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn  
 25                    50                    55                    60  
  
 Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr  
                     65                    70                    75                    80  
 30 Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln  
                     85                    90                    95  
  
 Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly  
                     100                    105                    110  
 35 Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met  
                     115                    120                    125  
  
 Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp  
 40                    130                    135                    140  
  
 Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu  
                     145                    150                    155                    160  
 45 Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly  
                     165                    170                    175  
  
 Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys  
                     180                    185                    190  
 50 Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val  
                     195                    200                    205  
  
 Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp  
 55                    210                    215                    220



Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr  
225 230 235 240

Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile  
5 245 250 255

Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val  
260 265 270

10 Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe  
275 280 285

Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe  
290 295 300

15 Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn  
305 310 315 320

Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met  
20 325 330 335

Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr  
340 345 350

25 Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe  
355 360 365

Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe  
370 375 380

30 Glu Phe Ile Phe Glu Arg Leu Lys Glu Leu Glu Asn Gln Met Leu Ala  
385 390 395 400

Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys  
35 405 410 415

Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val  
420 425 430

40 Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe  
435 440 445

Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro  
450 455 460

45 Arg Lys Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser  
465 470 475 480

His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr  
50 485 490 495

Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val  
500 505 510

55

Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser  
 515 520 525

Ser

5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 27 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CCGCCATGGA GACTTCCATT CTTACTC

27

(2) INFORMATION FOR SEQ ID NO: 18:

25

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 33 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

40 GCCGGATCCT CAAGATGACA AAGTTGGGAT GCT

33

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 1590 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

55

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Helianthus annuus*  
 (B) STRAIN: Zebulon

## 5 (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1590

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```

ATG GAG ACT TCC ATT CTT ACT CTC CTT CTT CTC TTG CTC TCA ACC CAA      48
Met Glu Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Ser Thr Gln
   1               5               10               15

15 TCT TCT GCA ACT TCC CGT TCC ATT ACA GAT CGC TTC ATT CAA TGT TTA      96
Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
           20               25               30

20 CAC GAC CGG GCC GAC CCT TCA TTT CCG ATA ACC GGA GAG GTT TAC ACT      144
His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
           35               40               45

25 CCC GGA AAC TCA TCT TTT CCT ACC GTC TTG CAA AAC TAC ATC CGA AAC      192
Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
           50               55               60

30 CTT CGG TTC AAT GAA ACT ACC ACA CCA AAA CCC TTT TTA ATC ATC ACA      240
Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr
           65               70               75               80

GCC GAA CAT GTT TCC CAC ATT CAG GCA GCT GTG GTT TGT GGC AAA CAA      288
Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln
           85               90               95

35 AAC CGG TTG CTA CTG AAA ACC AGA AGC GGT GGT CAT GAT TAT GAA GGT      336
Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly
           100              105              110

40 CTT TCC TAC CTT ACA AAC ACA AAC CAA CCC TTC TTC ATT GTG GAC ATG      384
Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met
           115              120              125

TTC AAT TTA AGG TCC ATA AAC GTA GAT ATC GAA CAA GAA ACC GCA TGG      432
Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp
           130              135              140

GTC CAA GCC GGT GCG ACT CTT GGT GAA GTG TAC TAT CGA ATA GCG GAG      480
Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu
50 145              150              155              160

AAA AGT AAC AAG CAT GGT TTT CCG GCA GGG GTT TGT CCA ACG GTT GGC      528
Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly
           165              170              175

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55

	GTT	GGT	GGG	CAT	TTT	AGT	GGT	GGT	GGG	TAT	GGT	AAT	TTG	ATG	AGA	AAA	576
	Val	Gly	Gly	His	Phe	Ser	Gly	Gly	Gly	Tyr	Gly	Asn	Leu	Met	Arg	Lys	
				180					185					190			
5	TAT	GGT	TTG	TCG	GTT	GAT	AAT	ATT	GTT	GAT	GCT	CAA	ATA	ATA	GAT	GTG	624
	Tyr	Gly	Leu	Ser	Val	Asp	Asn	Ile	Val	Asp	Ala	Gln	Ile	Ile	Asp	Val	
			195					200					205				
	AAT	GGC	AAG	CTT	TTG	GAT	CGA	AAG	AGT	ATG	GGT	GAG	GAT	TTG	TTT	TGG	672
10	Asn	Gly	Lys	Leu	Leu	Asp	Arg	Lys	Ser	Met	Gly	Glu	Asp	Leu	Phe	Trp	
		210					215					220					
	GCG	ATC	ACC	GGC	GGT	GGT	GGT	GTT	AGT	TTT	GGT	GTG	GTT	CTA	GCC	TAC	720
	Ala	Ile	Thr	Gly	Gly	Gly	Gly	Val	Ser	Phe	Gly	Val	Val	Leu	Ala	Tyr	
15	225				230						235					240	
	AAA	ATC	AAA	CTA	GTT	CGT	GTT	CCG	GAG	GTT	GTG	ACC	GTG	TTT	ACC	ATT	768
	Lys	Ile	Lys	Leu	Val	Arg	Val	Pro	Glu	Val	Val	Thr	Val	Phe	Thr	Ile	
					245					250					255		
20	GAA	AGA	AGA	GAG	GAA	CAA	AAC	CTC	AGC	ACC	ATC	GCG	GAA	CGA	TGG	GTA	816
	Glu	Arg	Arg	Glu	Glu	Gln	Asn	Leu	Ser	Thr	Ile	Ala	Glu	Arg	Trp	Val	
				260					265					270			
25	CAA	GTT	GCT	GAT	AAG	CTA	GAT	AGA	GAT	CTT	TTC	CTT	CGA	ATG	ACC	TTT	864
	Gln	Val	Ala	Asp	Lys	Leu	Asp	Arg	Asp	Leu	Phe	Leu	Arg	Met	Thr	Phe	
			275					280					285				
	AGT	GTC	ATA	AAC	GAT	ACC	AAC	GGT	GGA	AAG	ACA	GTC	CGT	GCT	ATC	TTT	912
30	Ser	Val	Ile	Asn	Asp	Thr	Asn	Gly	Gly	Lys	Thr	Val	Arg	Ala	Ile	Phe	
			290					295					300				
	CCA	ACG	TTG	TAC	CTT	GGA	AAC	TCG	AGG	AAT	CTT	GTT	ACA	CTT	TTG	AAT	960
	Pro	Thr	Leu	Tyr	Leu	Gly	Asn	Ser	Arg	Asn	Leu	Val	Thr	Leu	Leu	Asn	
35	305					310					315					320	
	AAA	GAT	TTC	CCC	GAG	TTA	GGG	TTG	CAA	GAA	TCG	GAT	TGT	ACT	GAA	ATG	1008
	Lys	Asp	Phe	Pro	Glu	Leu	Gly	Leu	Gln	Glu	Ser	Asp	Cys	Thr	Glu	Met	
					325					330					335		
40	AGT	TGG	GTT	GAG	TCT	GTG	CTT	TAC	TAC	ACG	GGC	TTC	CCC	AGT	GGT	ACT	1056
	Ser	Trp	Val	Glu	Ser	Val	Leu	Tyr	Tyr	Thr	Gly	Phe	Pro	Ser	Gly	Thr	
				340						345					350		
45	CCA	ACC	ACG	GCG	CTC	TTA	AGC	CGT	ACT	CCT	CAA	AGA	CTC	AAC	CCA	TTC	1104
	Pro	Thr	Thr	Ala	Leu	Leu	Ser	Arg	Thr	Pro	Gln	Arg	Leu	Asn	Pro	Phe	
			355					360					365				
	AAG	ATC	AAA	TCC	GAT	TAT	GTG	CAA	AAT	CCT	ATT	TCT	AAA	CGA	CAG	TTC	1152
50	Lys	Ile	Lys	Ser	Asp	Tyr	Val	Gln	Asn	Pro	Ile	Ser	Lys	Arg	Gln	Phe	
			370					375					380				
	GAG	TTC	ATC	TTC	GAA	AGG	ATG	AAA	GAA	CTT	GAA	AAC	CAA	ATG	TTG	GCG	1200
	Glu	Phe	Ile	Phe	Glu	Arg	Met	Lys	Glu	Leu	Glu	Asn	Gln	Met	Leu	Ala	
55	385						390					395				400	

TTC AAC CCA TAT GGT GGT AGA ATG AGT GAA ATA TCC GAA TTC GCA AAG 1248  
 Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys  
 405 410 415

5 CCT TTC CCA CAT AGA TCG GGT AAC ATA GCG AAG ATT CAA TAC GAA GTA 1296  
 Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val  
 420 425 430

10 AAC TGG GAG GAT CTT AGC GAT GAA GCC GAA AAT CGT TAC TTG AAT TTC 1344  
 Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe  
 435 440 445

15 ACA AGG CTG ATG TAT GAT TAC ATG ACT CCA TTT GTG TCG AAA AAC CCT 1392  
 Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro  
 450 455 460

20 AGA GAA GCA TTT TTG AAC TAT AGG GAT TTG GAT ATT GGT ATC AAC AGC 1440  
 Arg Glu Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser  
 465 470 475 480

CAT GGC AGG AAT GCT TAT ACT GAA GGA ATG GTT TAT GGG CAC AAA TAT 1488  
 His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr  
 485 490 495

25 TTC AAA GAG ACA AAT TAC AAG AGG CTA GTA AGT GTG AAG ACT AAA GTT 1536  
 Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val  
 500 505 510

30 GAT CCT GAC AAC TTC TTT AGG AAT GAG CAA AGC ATC CCA ACT TTG TCA 1584  
 Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser  
 515 520 525

35 TCT TG 1590  
 Ser 530

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 529 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

45

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Met Glu Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Ser Thr Gln  
 1 5 10 15

50 Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu  
 20 25 30

55 His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr  
 35 40 45

Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn  
 50 — 55 60

5 Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr  
 65 70 75 80

Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln  
 85 90 95

10 Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly  
 100 105 110

15 Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met  
 115 120 125

Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp  
 130 135 140

20 Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu  
 145 150 155 160

Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly  
 165 170 175

25 Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys  
 180 185 190

Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val  
 195 200 205

Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp  
 210 215 220

35 Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr  
 225 230 235 240

Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile  
 245 250 255

40 Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val  
 260 265 270

Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe  
 275 280 285

45 Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe  
 290 295 300

50 Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn  
 305 310 315 320

Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met  
 325 330 335

55

Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr  
 340 345 350  
 Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe  
 5 355 360 365  
 Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe  
 370 375 380  
 10 Glu Phe Ile Phe Glu Arg Met Lys Glu Leu Glu Asn Gln Met Leu Ala  
 385 390 395 400  
 Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys  
 405 410 415  
 15 Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val  
 420 425 430  
 Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe  
 20 435 440 445  
 Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro  
 450 455 460  
 25 Arg Glu Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser  
 465 470 475 480  
 His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr  
 485 490 495  
 30 Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val  
 500 505 510  
 Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser  
 35 515 520 525  
 Ser

## (2) INFORMATION FOR SEQ ID NO: 21:

40

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

45

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

50

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*
- (B) STRAIN: ecotype Columbia

55

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..350

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAGAAACTCG GAGACTTTCA CACAATGCCT AACCTCAAAC TCCGACCCCA AACATCCCAT 60  
CTCCCCCGCT ATCTTCTTCT CCGGAAATGG CTCCTACTCC TCCGTATTAC AAGCCAACAT 120  
10 CCGTAACCTC CGCTTCAACA CCACCTCAAC TCCGAAACCC TTCCTCATAA TCGCCGCAAC 180  
ACATGAATCC CATGTGCAAG CCGCGATTAC TTGCGGGAAA CGCCACAACC TTCAGATGAA 240  
15 AATCAGAAGT GGAGGCCACG ACTACGATGG CTTGTCATAC GTTACATACT CTGGCAAACC 300  
GTTCTTCGTC CTCGACATGT TTAACCTCCG TTCGGTGGAT GTCGACGTGG 350

## (2) INFORMATION FOR SEQ ID NO: 22:

20

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 278 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- 25 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

30

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana
- 35 (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- 40 (B) LOCATION: 2..278

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGCATGGATC TCCGCCGGAG CGACTCTCGG AGAGGTTTAT TATCGGATTT GGGAGAAAAG 60  
45 CAGAGTCCAT GGATTCCCCG CCGGAGTTTG ACCGACGGTT GGTGTTGGTG GGCATTTAAG 120  
CGGCGGTGGT TACGGTAACA TGGTGAGGAA GTTTGGATTA TCTGTGGATT ACGTTGAGGA 180  
50 TGCCAAGATC GTCGATGTAA ACNGTCGGGT TTTAGATCGG AAAGCAATGG GTGAGGATCT 240  
GTTCTGGGCG ATTACCGGTG GAGGAGGAGG TAGCGTAC 278



## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 345 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*
- (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..345

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

25 TGGACATATT AGCGGAGGAG GATTCGGTAC AATAATGAGG AAATACGGTT TAGCGTCTGA 60  
 TAACGTTGTG GACGCACGTT TGATGGATGT AAATGGGAAA ACTCTTGACC GGAAAACGAT 120  
 GGGAGAGGAT TTGTTTTGGG CGCTTAGAGG CCGTGGAGCT GCGAGTTTGT GCGTTGTCTT 180  
 30 GTCGTGGAAG GTTAAGCTTG CTAGGGTTCC TGAAAAGGTA ACTTGTTTCA TAAGTCAACA 240  
 TCCGATGGGA CCTAGCATGA ACAAGCTTGT TCATAGATGG CAATCCATAG GATCAAGANN 300  
 35 GCTAGACGAA GATTTATTCA TCAGAGTCAA TATTGACAAC AGTCT 345

## (2) INFORMATION FOR SEQ ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 695 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*
- (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..695

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GTTCGTTAAA ACCTATCCTN NANGGGCNAA AGNATATCAA AGNTTGNTTA NGNAACCCAA 60  
 NATTTCTGAA CTGGCCNCCT TCGGTGGTAT ATGNCNAAAN CCCTTGAATC TGCGNANCCN 120  
 10 ATTCCGCATA GAAACGGAAC CCTCTTCAAG ATTCTCTATT TACNCGAACT GNCTAGANNG 180  
 AATGACAAGA CATCGAGTAG NAAAATCAAC TGGATCAAAG AGATATACAA TTACATGGCG 240  
 15 CCTTATGTCT CAAGCAATCC AAGACAAGCA TATGTGAACT ACAGAGATCT AGACTTCGGA 300  
 CAGAACAAGA ACAACGCAAA GGTAACTTC ATTGAAGCTA AAATCTGGGG ACCTAAGTAC 360  
 TTCAAAGGCA ATTTTGACAG ATTGGTGAAG ATTAAAACCA AGGTTGATCC AGAGAACTTC 420  
 20 TTCAGGCACG AGCAGAGTAT CCCACCTATG CCCTACTAGA AGCTAGGTTC ATGAAACCAA 480  
 TAACATTATC AAAAATAAGR ATAAATGRTA ATTGTATACA ACATGATTCG KCTTTCTTTA 540  
 25 TTTTACAGACAA TGTGGACACT ACTCTAAANT AAAAWGTCNA TTTACCTTAA AAAAAAATA 600  
 ATCCCNNTA ANANAAAANT GGGGGGGCCN TTTTGGGGN TCCCGTTTT NGGACGGGGN 660  
 GCTTTNGGGG GGCTTGGNNT TTTTNGGN GCCCC 695

30

## (2) INFORMATION FOR SEQ ID NO: 25:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 495 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

35

## (ii) MOLECULE TYPE: cDNA to mRNA

40

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

45

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..495

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TCTGTTTTNA GGCAGAGCAG AGGAAGTTGT TGCTTTGCTT GGTAAGGAGT TTCCTGAATT 60  
 5 NAGTTTAAAG AAGGAGAACT GTTCGGAGAT GACTTGTTTT CAGTCAGCTT TATGGTGGGA 120  
 TAATCGTGTT AACCTACTC ANATTGATCC WAAAGTGTTT CTCGATCGGA ATCTTGATAG 180  
 AGCGAATTTT GGAAAGAGGA AATCGGATTA CGTTGCGAGT AAGATTCTTA GAGATGGGAT 240  
 10 TAAGYCTTTT TCCAAGARGA TGMCTGACCT GGGGAAAAYC GGGCTTGTTT TTAAWCCGTA 300  
 TGGTGGGAAA ATGGCGGAGG TTACGGTTAA CGCGACGCCG TTTCCNCACC GAAGCAAGCT 360  
 15 TTTTAAGATT CAGTACTCGG TGACTTNGCA AGAAAACCTCT NTCGAGATAG AGAAAGGGTT 420  
 TCTTGAATCA GGCTAACGTC CTTATAGGTT CATGACCGGG TTTTNNAGCA AGANCCCTGG 480  
 AATNCTTACT TNAAT 495

20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 204 base pairs  
 25 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

30

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

35 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana  
 (B) STRAIN: ecotype Columbia

(ix) FEATURE:

40 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

45 AAATTAAAAC AAATCAATGT TGATATTGAA TCCAATAGTG CTTGGTTTCA ACCTGGTGCT 60  
 ACGCTTGGTG AGCTTTACTA CAGAATTNCA GAGAAGAGCA AAATCCATGG ATTTCCNGCG 120  
 GGTNTNTNCA CAAGCNTAGG CATAGGTGGG TATATNANAG GCGGTGGATA CGGTACCTTG 180  
 50 ATGAGGAAGT ATGGTCTTNC GGGA 204

(2) INFORMATION FOR SEQ ID NO: 27:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 491 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

10

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana
- (B) STRAIN: ecotype Columbia

15

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..491

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAGATTTCTC GAGCAAGATA CTCCACTGAT GATCTTTGAG CCATTGGGTG GGAAAATCAG 60  
 CAAGATTTCA GAAACAGAAT CTCCATATCC ACACAGAAGA GGTAATCTGT ATAATATACA 120  
 25 GTACATGGTG AAATGGAAAG TGAATGANGT CGAGGAGATG AACAAACATG TCAGGTGGAT 180  
 GAGATCGTTA CACGATTACA TGA CTCCGTA TGTTTCTAAA TCGCCGAGAG GAGCTTATTT 240  
 30 GANTTACAGA GATCTTGATT TGGGCTCGAC CAAAGGGATT AACACGGGTT TCGGAGATGC 300  
 AAGGAAATGG NNGGGTGAGN CTTTTTTCAA AGGTAATTTT CAAGGGGTTA GGTTTTGGTT 360  
 AAAGGGGAGG TTTNNCCCAN CAAATTTTTT TTCAGGANCC GGCCANGNTT TTCCCCCCCC 420  
 35 TTTTTTTNGG NCCCCAATCN AAANCCCCGT TTTAAAAGGG GGGCCATTTT NTTTTTTNCA 480  
 NNTTAAAAGG G 491

40 (2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 407 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA to mRNA

50

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

55

- (A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

(ix) FEATURE:

(A) NAME/KEY: CDS

5 (B) LOCATION: 3..407

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

10 ATTTGTTTCGT GAGGTAACT TTGACTTTAG TCAACGGTAC GAAGCCTGGT GAGAATACGG 60  
TTTTAGCGAC TTCATTGGG ATGTATTTAG GCCGGTCGGA TAAGCTGTTG ACCGTNATGA 120  
ACCGGGATTT CCCGGAGTTG AAGCTGAAGA AAACCGATTN TACCGAGATG AGATGGATCG 180  
15 ATTCGGTTCT GTTTTGGGAC GATTATCCGG TTGGTACACC GACTTCTGTG CTACTAAATC 240  
CGCTAGTCGC AAAAAAGTTG TTCATGAAAC GAAAATCGGA CTACGTGAAG CGTCTNATTT 300  
TCGAGAACCC GATCTCNGT TTGATACTCA AGAAATTTGT AGAGGTTNNG AAAGTTAAAA 360  
20 TNAATTTGGA TCCGCATTNN GGNANNNATG GTGAAACCCC NNGTTNT 407

(2) INFORMATION FOR SEQ ID NO: 29:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

35 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

40

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 3..360

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ACGGCGTCGT ATTGGCCTAC AAAATAAACC TTGTTGAAGT CCCAGAAAAC GTCACCGTTT 60  
TCAGAATCTC CCGGACGTTA GAACAAAATG CGACGGATAT CATTACCGG TGGCAACAAG 120  
50 TTGCACCGAA GCTTCCCGAC GAGCTTTTCA TAAGANCAGT CATTGACGTA NAAACGGCAC 180  
TGTTTCATNN CTCAAAAGAC CGTCAGACAA CATTATAGC AATGTTTCTA GGAGACACGN 240  
55 CAACTCTACT GTCGATATTA AACCGGAGAT TCCCAGAATT GGGTTTGGTC CGGTCTGACT 300

GTACCGNAAC AAGCNNNTGG ATCCAATCTG TGCTATTTTT GGGACAAATA TCCCAGGTTG 360

(2) INFORMATION FOR SEQ ID NO: 30:

5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 427 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

15 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Arabidopsis thaliana  
 (B) STRAIN: ecotype Columbia

20 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 3..427

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TCTTCACTGT CACCAAAACG TTAGAACAAG ACGCAAGATT GAAGACTATT TCTAAGTGGC 60  
 AACAAATTTT ATCCAAGATT ATTGAAGAGA TACACATCCG AGTGGTACTC AGAGCAGCTG 120  
 30 GAAATGATGG AAACAAGACT GTGACAATGA CCTACCTAGG TCAGTTTCTT GGCGAGAAAG 180  
 GCACCTTGCT GAAGGTTATG GAGAAGGCTT TTCCAGAACT AGGGTTAACT CAAAAGGATT 240  
 35 GTACTGAAAT GAGCTGGATT GAAGCCGCCC TTTTCCATGG TGGRTTTCCA ACAGGKTCTC 300  
 CTATTGAAAT TTTGCTTMAG CTCAAGTCGC CTYTAGGAAA AGRTTWCTTC AAAGCAACGK 360  
 CGGATTTTCGT TAAAGAACCT WTTCTGTGA TAGGGCTCAA AGGAATATTC AAAAGATTGA 420  
 40 TTGAAGG 427

(2) INFORMATION FOR SEQ ID NO: 31:

45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 437 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

55 (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(B) STRAIN: ecotype Columbia

## 5 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..437

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

10 GTTGTACTAT CATNGAAGAT TAAGTTAGTC GATGTTCCGT CCACGGTCAC CGNGTTTAAA 60  
 GTCCAGAAAC ATNAGGAGAA AGAGGCCGTT AGGNTCATCA ACAAGTGGCA GTATGTTGCG 120  
 15 GATAAGGTCC CTGAAGATCT TTTCATCAGC GCAACGTTGG NGAGATCAAA CGGAAACTCT 180  
 GTGCAGGCTT TGTTTACTGG ACTCTATCTT GGNCCGGTGA ATAATNTCTT GGCCTTGATG 240  
 GAAGAAAAGT TTCCAGANTT AGGTCTTGAT ATCCAAGNCT GCACAGAGAT GAGTTGGGCT 300  
 20 GAATCTGCAC TCTGGTNTNC TGNTTTCNCT AAAGGAGAGN CTCCTTGGGT GTTCNCGCG 360  
 GATCGGNAGC GGNCAATTTN TGGNCTTTCA AGGGGAAAGN CGGCTTTTTN CAAGAACCCG 420  
 25 NTACCCGGGG TTCAATT 437

## (2) INFORMATION FOR SEQ ID NO: 32:

## (i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 441 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

40

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

## (ix) FEATURE:

45

(A) NAME/KEY: CDS

(B) LOCATION: 1..441

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

50 GCGGACCCTA TAGATCANNA TGTGCTACTG ANAGAAGAGG AAGCCAAGAA CAAGCCGGAG 60  
 ACAGATAAAT ATCTGAAATG GGNCGATANC GTTTACGAAT TTATGACNCC ATATGTTTCG 120  
 AAATCTCCAA GAGGAGCTTA TGTCAATTTC AAGGATATGG ATTTGGGTAT GTATCTTGGA 180  
 55

AAGAAGAAGA CAAAGTACGA GGAAGGAAAG AGTTGGGGAG TGAAGTATTT CAAGAACAAT 240  
TTCGAGAGAT TGGTGAGAGT GAAGACTAGG GTTGATCCAA CAGATTTCTT CTGCGATGAA 300  
5 CAGAGCATTC CTCTGGTGAA CAAAGTTACC TGAAGATATC ATTTGAAGTT TTTTATTAGT 360  
CCCTTTTCTC TGTGAAATCA TCTGTGCGTG TTGAATATTA TCGTCAAGT GTGTAACCTA 420  
TGTGTGTGAT TGTGAATTGT G 441

10

(2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:  
15 (A) LENGTH: 502 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA  
20
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- 25 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: *Arabidopsis thaliana*  
(B) STRAIN: ecotype Columbia
- (ix) FEATURE:  
30 (A) NAME/KEY: CDS  
(B) LOCATION: 2..502

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

35 CTGGCTTAAC ACAACGTCGT TTTGGGCCAA TTACCCGGCG GGTACACCCA AGAGCATCCT 60  
TCTAGATAGG CCTCCGACGA ATTCAGTGTC ATTTAAGAGT AAATCGGATT TTGTCAAAAA 120  
ACCAATACCC AAAAAAGGTT TAGAGAAGCT TTGGAAGACA ATGTTTAAAT TCAACAGTAG 180  
40 CGTCTCGTTG CAATTCAACC CTTACGGTGG AGTGATGGAC CGGATTCCGG CAACGGCCAC 240  
CGCTTTTCCT CATCGGAAAG GAAACTTGTT CAAGGTTCAA TACNCTACGA TGTGGTTTGA 300  
45 CGCAAACGCC ACACAGAGTA GCCNGGCTAT GATGAATGAG CTTTTTGAGG TGGCGGGACC 360  
GTACGTGNGT CAAGTAAACC CGAGANANGG CTCCTTTTAA NTTAGAGNC CATCGNTNTT 420  
NGGAGCAANN CCAAGTGGGG GGGNCCAACC GGGGGNTNAA ANCNAGNTC TTNGGGGGCC 480  
50 CAGAATTTC TTNNGGGAAT TT 502

(2) INFORMATION FOR SEQ ID NO: 34:

55



(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 400 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: double  
5       (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO  
10

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
    (A) ORGANISM: Arabidopsis thaliana  
15       (B) STRAIN: ecotype Columbia

(ix) FEATURE:  
    (A) NAME/KEY: CDS  
    (B) LOCATION: 2..400  
20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

    NGGGAATTGC NCGAGGNAAG TTGTACCCAA TTCCTGGACC ACCATTGGTT TCCCAAGAAN   60  
25   CCCGAGACAA CCGTTTTTCA ATNACCGTGA TGTTGATTG GGTATTAATT CTCATAATGG 120  
    TAAAATCACT AGTTATGTGG AAGGTAAACG TTACGGGAAG AAGTATTTTCG CAGGTAATTT 180  
30   CGAGAGATTG GTGAAGATTA AGACGAGAGT TGATAGTGGT AATTTCTTTA GGAACGAACA 240  
    GAGTATTCCT GTGTTACCAT AAGTGATTTT ATTTGATTAT TGGTTAGTGA AATTTGTTGT 300  
    TGTATAATGA TTATATGTCG TATTTTATT TATTATTAGT AATTTATAAA GTTTGATATT 360  
35   AAATACAAAT AGTATAATAA GATAGTTTCT TTAGTAAAA                   400

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:  
40       (A) LENGTH: 383 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: double  
          (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA  
45

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO  
50

(vi) ORIGINAL SOURCE:  
    (A) ORGANISM: Arabidopsis thaliana  
    (B) STRAIN: ecotype Columbia  
55

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..383

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CAACTCTAAT GGGAACACCT ACTTCGATCG AATGTCGATG GGGGAAGAGC TTTTCTGGGC 60  
 GGTTCGAGGA GGTGGAGCCG CGAGTTTCGG CATCGTGATG GGATACAAAA TCCGGTTGGT 120  
 10 TCCGGTTCCG GAGAAAGTTA CGGTTTTTAG CGTCGGAAAA ACCGTCGGAG AAGGAGCCGT 180  
 TGATCTTATA ATGAAGTGGC AGAACTTCTC TCATAGTACG GNTCGGAATT TTTTTGTGAA 240  
 15 GCTGANTTTT GANTTTAGTC AACGGTGCAA AGCCGGGTGA AAAAAAGGTT TTAGNGNCTT 300  
 TCANTTTGGN TGNAANCTTG GGGGTTTTAT NAGAACGGTT AACCGGGATT NANCCCGNGT 360  
 TTTCCCGGGG TTAAACCTT NGG 383

20

## (2) INFORMATION FOR SEQ ID NO: 36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 354 base pairs
- 25 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

30

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## 35 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana
- (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

- 40 (A) NAME/KEY: CDS
- (B) LOCATION: 1..354

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

45 ATCAATGTTC TTAATAACG TACACGAGCA TCGTTGGCTT TCAAGGCTAA ATCTGATTTT 60  
 NTTCAAGAAC CGATNCCTAA AACCGCGATT TCGAAGCTTT GGAGACGGTT GCAAGAACCG 120  
 GAAGCAGAGC ATGCTCAGCT AATTTCACN CCATTTGGTG GTAAAATGAG TNAGATTGCA 180  
 50 GATTACGAAA CACCATTTC GCATAGGAAG GGGAATATAT ATNAGATTCA GTACTTGAAT 240  
 TACTGGAGAG GAGACGTGAA AGAGAAGTAT ATTGAGATNG GTGGAGGAGA GTTTACGGTT 300  
 55 GNTATNAGTA AGTTTTTTGG CGAAGTNTNC CNAGAGGNGN CTTNNTNTAA ACCT 354

## (2) INFORMATION FOR SEQ ID NO: 37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana
- (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..403

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

25 TTTTITAGTA CACTAATAAT CAAATGGAAT GAGAAATGAA GCCACAAAAG TATCTGCAAT 60  
 CAAAATATCC TGCTATCTCC ATCTCAAGCT CTCAATAGTA TCCTCTCCGA AAGTGAAATC 120  
 AACATTTCAA ACTCTATTTT TTGGTGGAAT CGATAGACTG ATTCCTCTGA TGAACCAGAA 180  
 30 GTTTCCTGAA CTCGGCTTAC GATCTCAAGA CTGTTCGGAA ATGAGCTGGA TCGAATCGAT 240  
 AATGTTCTTC AACTGGAGAT CAGGACAGCC GTTAGAGATT TTGCTCAACA GAGACCTAAG 300  
 35 GATTCGAGGA TCAGTATTTT AAAGCAAAGT CAGGATTATG GTTCAAAAAC CCGTTCCTGA 360  
 AAACGTTTTT CGAAGAGGTA TCCAAGGGGT TTCTCGAGCA AGT 403

## (2) INFORMATION FOR SEQ ID NO: 38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 260 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana
- (B) STRAIN: ecotype Columbia

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..260

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GAGATGAGTT GGATTAANTC TGTACTCTGG TTTGCTGATT TCCCTAAAGG AGAATCTCTT 60  
 NGTGTCTCA CGAATCGTAA GCGTACATCT CTATCTTTNA AAGGCAAAGA TGATTTTATC 120  
 10 CAAGAACCGA TACCCGAGGC TGCAATTNAA GAGATATGGA GGCGATTAGA AGCCCCNAG 180  
 GCTCGGCTTG GAAAGATCAT ATTAACCTCA TTTGGTGGGA AAATNAGTGA AATGGCAGAG 240  
 15 TACGTANCAC CATTCCCACA 260

## (2) INFORMATION FOR SEQ ID NO: 39:

## (i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 605 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

30 (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana  
 (B) STRAIN: ecotype Columbia

## 35 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..605

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

40 CTCTTGCATA TTCGCTGCAA GGATGGGAAA TTCAAACCA CTCCTACAA TTTTTGTAT 60  
 TATAGTTTCA GTCTTGATTT TTTAATTCTA TTGCATAACA CCAACTTCTT CATCAGCCTC 120  
 45 CATCCAAGAT CAATTCATAA ACTGTGTCAA AAGAAACACA CATGTTTCTT TTCCACTCGA 180  
 GAAAACGTTA TTCACCCCTG CGAAAAACGT CTCTTTGTTC AACCAAGTCC TTGANTCGAC 240  
 GGCTCAAAAT CTCCAGTTCT TGGCAAAATC CATGCCTAAA CCGGGRTTCA TATTCAGACC 300  
 50 GATTACACCAG TCTCAAGTCC AAGSTTCCAT CATTTGTTCA AMGRAACTCG GGNTTCATTT 360  
 TNGTGTGTTGA NGTGGCGGTC ACGATTTTCG AGGCCTTTGT NTTTATGTTT CACGGTTTGA 420  
 55 AAAAACCGTT TATATTACTC GGCCTGTCAA ANTTGNANNC AAAATCANAT GTTGGATATT 480

GNATTCCAAA TAGGTNCTTG GGGTNAACCT GGTGGCTANC GTTTGGTGAG CTTTACTTT 540  
 CAAGAATTG CANGNGGANG TGCAAAGATT CCATGGGATT TCCCGGGGGG TTTNTTGCAC 600  
 5 AATGT 605

(2) INFORMATION FOR SEQ ID NO: 40:

- 10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 464 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- 15 (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- 20 (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: *Arabidopsis thaliana*  
 (B) STRAIN: ecotype Columbia
- 25 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 2..464

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

AACACAAAAC TCTTCCATTT GGCTTCTCTC TTGCATATTC GTTGCAAGGA TGGGAAATTC 60  
 AAAACCACTC CCTACAATTN CTTGTATTAT CGTTTCAGTC TTGTATTTTN NATTCTATTG 120  
 35 CATAACACCA ACTTCTTCAT CAGCCTCCAT CCAAGNTCAA TTCATAAACT GTGTCAAAAAG 180  
 GAACACACAT GTTCTTTTTC CACTCGAGNA AACGGTATTC ACTCCTGCGG AAAACGGCTC 240  
 40 TTTTATTCAA CGGGTCCNTG AATCGACGGG TCAAAATCTC CAGTTCTTGG NAAAATCCAT 300  
 GNCTAAACCG GGGTTCATAT TCAGGCCGGT TCACCAGTCT CAAGTCCAAG NTTCCATCAT 360  
 TTGTTCAAAG GAACTCGGGA TTCATTTCCG CGNTAGAAGT GGCGGGCANN GGTTCGGGG 420  
 45 CCTGTCTNTT GNTTANGGN AGGAAAACCG GTTNTATTNC TCGG 464

(2) INFORMATION FOR SEQ ID NO: 41:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 386 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- 55

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

5 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(B) STRAIN: ecotype Columbia

10 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..386

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

TCGGGAGCCC ANGNTAAATT ANNTGAAAAT GGGGNCGNAT ANCCGTTTAC NGAATTTTAT 60

GACNCCCAAT ATGTTTCGAA ATCTCAAAGA NNGGGANCTT ATGTCAATTT CAAGGATATG 120

20 GATTTGGGTA TGTATCTTGG AAAGNAGAAG ACAAAGTACG AGGAAGGAAA GAGTTGGGGA 180

GTGAAGTATT TCAAGAACAA TTTGAGAGA TTGGTGAGAG TGAAGACTAG GGTGATCCN 240

25 ACAGATTTTCN TCTGCGATGA ACAGAGCATT CCTCTGGTGN ACAAAGTTAC CTGAAGATAT 300

CATTTGAAGT TTTTATTAG TCCCTTTTCT CTGTGAAATC ATCTGTGCGT GTTGAATANT 360

ATGCGTCAAG TGTGTAACCT ATGTGT 386

30 (2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 377 base pairs

35 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

40 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(B) STRAIN: ecotype Columbia

45 (ix) FEATURE:

(A) NAME/KEY: CDS

50 (B) LOCATION: 1..377

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

55 TACCATAGGG AGGTGGTGNA AGATTTTGTA TGTAGNCTTA GGGGAAGGCG AGTAGTATGG 60

TGGTGGTGGG GAGCTGTAAA CGTATGGTGG TGGTGGAGAT TTGTATGTGG GCTGGTTAAC 120  
 TTCATTGAAG CTAAAATCTG GGGACCTAAG TACTTCAAAG GCAATTTTGA CAGATTGGTG 180  
 5 AAGATTAAAA CCAAGGTTGA TCCAGAGAAC TTCTTCAGGC ACGAGCAGAG TATCCACCT 240  
 ATGCCCTACT AGAAGCTAGG TTCATGAAAC CAATAACATT ATCAAAAATA AGAATAAATG 300  
 ATAATTGTAT ACAACATGAT TCGTCTTTCT TTATTTTCAGA CAATGTGGAC ACTACTCTAA 360  
 10 ATAAAATGTC ATTTACC 377

(2) INFORMATION FOR SEQ ID NO: 43:

- 15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 377 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear  
 20 (ii) MOLECULE TYPE: cDNA to mRNA  
 (iii) HYPOTHETICAL: NO  
 25 (iii) ANTI-SENSE: NO  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Arabidopsis thaliana  
 (B) STRAIN: ecotype Columbia  
 30 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..377  
 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

TACCATAGGG AGGTGGTGNA AGATTTTGTA TGTAGNCTTA GGGGAAGGCG AGTAGTATGG 60  
 TGGTGGTGGG GAGCTGTAAA CGTATGGTGG TGGTGGAGAT TTGTATGTGG GCTGGTTAAC 120  
 40 TTCATTGAAG CTAAAATCTG GGGACCTAAG TACTTCAAAG GCAATTTTGA CAGATTGGTG 180  
 AAGATTAAAA CCAAGGTTGA TCCAGAGAAC TTCTTCAGGC ACGAGCAGAG TATCCACCT 240  
 45 ATGCCCTACT AGAAGCTAGG TTCATGAAAC CAATAACATT ATCAAAAATA AGAATAAATG 300  
 ATAATTGTAT ACAACATGAT TCGTCTTTCT TTATTTTCAGA CAATGTGGAC ACTACTCTAA 360  
 ATAAAATGTC ATTTACC 377

50 (2) INFORMATION FOR SEQ ID NO: 44:  
 (i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 346 base pairs  
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..346

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

20 GAGCTGTGGA TATGGTCACA AATGGCAATC GGTGGTCCG AAAACTGATC CGAATCTTTT 60  
TATGAGAATN TTGATTCAAC CAGTGACGAG GAAGAAGGTA AAGACTGTGA GAGCTTCTNT 120  
GGTTGCCCTN TTTTNAGGCN AGACAGATGA AGTTTTTGCT TTCCTTAGTA AGGAGTTTCC 180  
25 TGAATTGGGT TTAAAGAAGG AGAATTNTTC GGAGATGACT TGGTTTCANT CTGCTTTATG 240  
GTGGGACAAT CGTCTTAATG CTAATCAGGT TGATCCTAAA GTNTTCTTG ATCGGAATCT 300  
30 CGATACCTCG AGTTTCGGTA AGAGGAAATC GGATTACGTC GCGACT 346

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 261 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..261

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:



ATGGGGTGAG ACTTATTTCA AAGGTAATTT CAAGAGATTA GGTTTGGTTA AAGGGAAGNT 60  
 TGATCCAACA AATTTCTTCA GGAACGAACA GAGTATTCCT CCTCTGTTTT GAGTCCTCAA 120  
 5 TACAAAACCA GATATAAAG ATGTCATTTC ATTTTTTCAA TTATAATAGA TAATGTAAGT 180  
 TTCTGCTACA ATTGTAAGG TGAGATGTAC CCAATACGGT TTAAGCGGAC CGAGAATAGT 240  
 CAATTCAAAG ACCAAATTCT G 261

10

(2) INFORMATION FOR SEQ ID NO: 46:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 478 base pairs
  - 15 (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- 20 (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- 25 (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Arabidopsis thaliana
  - (B) STRAIN: ecotype Columbia
- (ix) FEATURE:
  - 30 (A) NAME/KEY: CDS
  - (B) LOCATION: 1..478

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

35 GCTCAAAGGA CTAACCATGA AAACCTCCTC AAGTGTCTCT CTCACCGANT CAACGAGGAC 60  
 GACTCAAGAN TTATACACAC ATCAAAAGAT CCTTCGTATT TNTCAATCTT GATTTCTTCC 120  
 ATACAAAATC CAAGTTTCTC TGTTCCTGAA ACACCTAAAC CGGTTTCAAT CATCACTCCG 180  
 40 GTTCAAGCCA CCGATGTTCA ATCTACGNTT AAATNCGCAC GGNCTTCACG GGTATACACA 240  
 ATCAGGGCTA GGAGTGGTNG TCATGACTAC GGAGGTTTAT CTTTACATTG GCTTAAAAAN 300  
 45 CANNCCGTTT GTTNNTCATT GATTTNNAGA AATCTTCCGG GCTTATTTAA CATNTAAGAT 360  
 GTTTGATAAN CCGGNNCCNG TTTGGGGTTC AAATCCCGGT GGCTTACAAA NTTNGGGGGA 420  
 ATTGTNCCTA TGAGGTTTGG AAAATTAANG CAAAATNTTT TGGGCCTTCC CGGCCGGT 478  
 50

(2) INFORMATION FOR SEQ ID NO: 47:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 579 base pairs
  - 55 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

5

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

10

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(B) STRAIN: ecotype Columbia

(ix) FEATURE:

15

(A) NAME/KEY: CDS

(B) LOCATION: 2..579

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

20 GGCCGTTAGG ATCATCAAGA AATGGCAATA TGCTGCAGAT AAGGTTCTCTG ATGATCTTTT 60  
CATTAGGACA ACATTGGAGA GATCAAACAA GAACGCAGTA CACGCTTTGT TCACTGGACT 120  
ATATATTGGT CCGGTGAACA ATCTATTGGC GTTGATGGAA GAAAAGTTTC CGGAAGTAGG 180  
25 TCTTGAGAAA GAAGGTGTG AAGAGATGAG TTGGATTGAG TCTGTACTCT GGTTCGCTGA 240  
TTTCCCTAAA GGAGAATCTC TTGGTGTCTC CACGAATCGT GAGCGTACAT CTCTATCTTT 300  
30 CAAAGGCAAA GATGATTTTG TCCAAGAACC GATACCCGAG GGTGCAATTC AAGAGATATG 360  
GAGGCGATTA GAAGCCCCCG AGGCTCGGCT TGGAAAGATC ATATTAATCT CATTTGGGTG 420  
NGGNAAAATG AGTGAAATGG CAGAGNCCGA ACCACCAATT CCCACANNCG AGGGAGGGGA 480  
35 ACCCCTNTGN GGNTCAGAAT GTGGTTCCTG GNNNNNAAGN GGGNGCCAGN ACCAANCCGG 540  
GNCNGTAAAN CNTGNAATGG GCCNAACCCG TNCCGGATT 579

40 (2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 252 base pairs

(B) TYPE: nucleic acid

45

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

50

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

55

(A) ORGANISM: *Oryza sativa*

(B) STRAIN: Nipponbare, subsp. japonica

(D) DEVELOPMENTAL STAGE: etiolated shoot (8 days old)

(ix) FEATURE:

5

(A) NAME/KEY: CDS

(B) LOCATION: 3..252

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

10 TGTCCCTGGAA GGTCCGCCTC GTGCAGGTTN CGACGACGGT GACGGTGTTT GTCGTCCGGA 60  
 GGAACGTCGA CCAGGGCGCC GCNGACGTCG TCGCCAGATG GCAAGACGTC GCGCCGAGCC 120  
 TCCCTCCCGA GCTCACCATA CGGGTGATCG TNCGAGGGCA GCGCGCCACG TTCCAGTCGC 180  
 15 TGTACCTCGG CTCGTGCGCC GACCTGGTGC CGACGATGAG CAGCATGTTC CCGGAGCTCG 240  
 GGATGACGAT TG 252

20 (2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30 (iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactuca sativa

(B) STRAIN: lollo bionda

35

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12

40 (D) OTHER INFORMATION: /label= Ambiguous  
 /note= "Xaa = Cys or Ser"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 20..21

45 (D) OTHER INFORMATION: /label= ambiguous  
 /note= "Xaa-Xaa probably is Ser-Phe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

50 Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Xaa Leu Asn Asn Arg  
 1 5 10 15  
 Ala Asp Pro Xaa Xaa  
 20  
 55

## (2) INFORMATION FOR SEQ ID NO: 50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactuca sativa
- (B) STRAIN: lollo bionda

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /label= ambiguous  
/note= "Xaa = probably Ser"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /label= unknown

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /label= ambiguous  
/note= "Xaa = probably Ser"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /label= ambiguous  
/note= "Xaa = probably Trp"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 24
- (D) OTHER INFORMATION: /label= ambiguous  
/note= "Xaa = probably Tyr"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Xaa Ile Xaa Val Xaa Ile Glu Asp Glu Thr Ala Xaa Val Gln Ala Gly  
 1                      5                      10                      15  
 Ala Thr Leu Gly Glu Val Tyr Xaa  
                     20

## (2) INFORMATION FOR SEQ ID NO: 51:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactuca sativa
- (B) STRAIN: lollo bionda

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Ala Asp Pro Ser Phe Pro Leu Ser Gly Gln Leu Tyr Tyr Pro  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO: 52:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

ACTTCTACTT CTATTATTGA TAGGTTTACT CA 32

## (2) INFORMATION FOR SEQ ID NO: 53:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 405 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactuca sativa
- (B) STRAIN: lollo bionda

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..405

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACT TCT ACT TCT ATT ATT GAT AGG TTT ACT CAA TGT CTA AAC AAC CGA 48  
 Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg  
 1 5 10 15  
 10 GCT GAC CCT TCT TTC CCG CTC AGT GGA CAA CTT TAC ACT CCC GAT AAC 96  
 Ala Asp Pro Ser Phe Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn  
 20 25 30  
 15 TCC TCT TTT CCA TCC GTC TTG CAA GCT TAC ATC CGG AAC CTC CGA TTC 144  
 Ser Ser Phe Pro Ser Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe  
 35 40 45  
 AAT GAA TCC ACG ACT CCC AAA CCC ATC TTA ATC ATC ACC GCC TTA CAC 192  
 20 Asn Glu Ser Thr Thr Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His  
 50 55 60  
 CCT TCA CAC ATT CAA GCA GCT GTT GTG TGC GCC AAA ACA CAC CGC CTG 240  
 Pro Ser His Ile Gln Ala Ala Val Val Cys Ala Lys Thr His Arg Leu  
 25 65 70 75 80  
 CTA ATG AAA ACC AGA AGC GGA GGC CAT GAT TAT GAG GGG CTT TCC TAT 288  
 Leu Met Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr  
 85 90 95  
 30 GTG ACC AAT TCG AAC CAA CCC TTT TTT GTT GTT GAC ATG TTC AAC TTA 336  
 Val Thr Asn Ser Asn Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu  
 100 105 110  
 35 CGC TCC ATA AAC GTG AGT ATT GAA GAT GAA ACT GCA TGG GTC CAA GCC 384  
 Arg Ser Ile Asn Val Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala  
 115 120 125  
 GGC GCC ACC CTC GGA GAA GTT 405  
 40 Gly Ala Thr Leu Gly Glu Val  
 130 135

## (2) INFORMATION FOR SEQ ID NO: 54:

45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 135 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg  
 55 1 5 10 15

25 (2) INFORMATION FOR SEQ ID NO: 55:

30

35

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

25

40

(2) INFORMATION FOR SEQ ID NO: 56:

45

50

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

23

## (2) INFORMATION FOR SEQ ID NO: 57:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1981 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

10

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

15

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactuca sativa  
 (B) STRAIN: lollo bionda

## (ix) FEATURE:

20

- (A) NAME/KEY: CDS  
 (B) LOCATION: 7..1626

## (ix) FEATURE:

25

- (A) NAME/KEY: unsure  
 (B) LOCATION: replace(372, "g")

## (ix) FEATURE:

30

- (A) NAME/KEY: unsure  
 (B) LOCATION: replace(379, "g")

## (ix) FEATURE:

35

- (A) NAME/KEY: unsure  
 (B) LOCATION: replace(786, "t")

## (ix) FEATURE:

- (A) NAME/KEY: unsure  
 (B) LOCATION: replace(1105..1106, "ga")  
 (D) OTHER INFORMATION: /note= "also possible "gg" and "aa"

40

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

45 ACAAAA ATG GCA ATT ACC TAT TCT TTC AAC TTC AAA TCT TAT ATT TTT 48  
 Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe  
 1 5 10

50 CCT CTC CTC CTT GTC TTG CTC TCT ACC CAT TCA TCA GCG ACT TCA ACT 96  
 Pro Leu Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr  
 15 20 25 30

TCC ATT ATA GAT CGC TTC ACC CAA TGT CTA AAC AAC CGA GCT GAC CCT 144  
 Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro  
 35 40 45

55



	TCT	TTC	CCG	CTC	AGT	GGA	CAA	CTT	TAC	ACT	CCC	GAT	AAC	TCC	TCT	TTT	192
	Ser	Phe	Pro	Leu	Ser	Gly	Gln	Leu	Tyr	Thr	Pro	Asp	Asn	Ser	Ser	Phe	
				50					55					60			
5	CCA	TCC	GTC	TTG	CAA	GCT	TAC	ATC	CGG	AAC	CTC	CGA	TTC	AAT	GAA	TCC	240
	Pro	Ser	Val	Leu	Gln	Ala	Tyr	Ile	Arg	Asn	Leu	Arg	Phe	Asn	Glu	Ser	
			65					70					75				
10	ACG	ACT	CCC	AAA	CCC	ATC	TTA	ATC	ATC	ACC	GCC	TTA	CAC	CCT	TCA	CAC	288
	Thr	Thr	Pro	Lys	Pro	Ile	Leu	Ile	Ile	Thr	Ala	Leu	His	Pro	Ser	His	
		80					85					90					
15	ATT	CAA	GCA	GCT	GTT	GTG	TGC	GCC	AAA	ACA	CAC	CGC	CTG	CTA	ATG	AAA	336
	Ile	Gln	Ala	Ala	Val	Val	Cys	Ala	Lys	Thr	His	Arg	Leu	Leu	Met	Lys	
	95					100					105				110		
20	ACC	AGA	AGC	GGA	GGC	CAT	GAT	TAT	GAG	GGG	CTT	TCC	TAT	GTG	ACC	AAT	384
	Thr	Arg	Ser	Gly	Gly	His	Asp	Tyr	Glu	Gly	Leu	Ser	Tyr	Val	Thr	Asn	
				115					120					125			
25	TCG	AAC	CAA	CCC	TTT	TTT	GTT	GTT	GAC	ATG	TTC	AAC	TTA	CGC	TCC	ATA	432
	Ser	Asn	Gln	Pro	Phe	Phe	Val	Val	Asp	Met	Phe	Asn	Leu	Arg	Ser	Ile	
			130						135					140			
30	AAC	GTG	AGT	ATT	GAA	GAT	GAA	ACT	GCA	TGG	GTC	CAA	GCT	GGT	GCG	ACT	480
	Asn	Val	Ser	Ile	Glu	Asp	Glu	Thr	Ala	Trp	Val	Gln	Ala	Gly	Ala	Thr	
			145					150					155				
35	CTT	GGT	GAA	GTC	TAC	TAC	CGA	ATA	GCA	GAG	AAA	AGC	AAC	AGT	CAT	GCT	528
	Leu	Gly	Glu	Val	Tyr	Tyr	Arg	Ile	Ala	Glu	Lys	Ser	Asn	Ser	His	Ala	
		160					165					170					
40	TTT	CCG	GCT	GGC	GTT	TGC	CCT	ACT	GTT	GGA	GTT	GGT	GGC	CAT	TTT	AGT	576
	Phe	Pro	Ala	Gly	Val	Cys	Pro	Thr	Val	Gly	Val	Gly	Gly	His	Phe	Ser	
	175					180				185					190		
45	GGT	GGT	GGT	TAT	GGT	AAC	TTG	ATG	GGA	AAA	TAC	GGC	CTT	TCT	GTT	GAC	624
	Gly	Gly	Gly	Tyr	Gly	Asn	Leu	Met	Gly	Lys	Tyr	Gly	Leu	Ser	Val	Asp	
				195					200					205			
50	AAT	ATT	GTC	GAT	GCT	CAG	TTA	ATC	GAT	GTG	AAT	GGT	AAA	CTT	CTG	AAT	672
	Asn	Ile	Val	Asp	Ala	Gln	Leu	Ile	Asp	Val	Asn	Gly	Lys	Leu	Leu	Asn	
			210						215					220			
55	CGG	AAA	TCA	ATG	GGT	GAA	GAT	CTT	TTT	TGG	GCC	ATC	ACA	GGT	GGT	GGT	720
	Arg	Lys	Ser	Met	Gly	Glu	Asp	Leu	Phe	Trp	Ala	Ile	Thr	Gly	Gly	Gly	
			225					230					235				
60	GGT	GTC	AGC	TTT	GGT	GTG	GTT	GTA	GCG	TAC	AAG	ATC	AAA	CTG	GTT	CGT	768
	Gly	Val	Ser	Phe	Gly	Val	Val	Val	Ala	Tyr	Lys	Ile	Lys	Leu	Val	Arg	
		240					245					250					
65	GTT	CCT	ACC	ACT	GTG	ACC	GTT	TTT	AAC	GTA	CAA	AGA	ACA	TCC	GAG	CAG	816
	Val	Pro	Thr	Thr	Val	Thr	Val	Phe	Asn	Val	Gln	Arg	Thr	Ser	Glu	Gln	
	255					260					265				270		

	AAC CTA AGC ACC ATA GCC CAC CGA TGG ATA CAA GTT GCG GAT AAG CTC	864
	Asn Leu Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu	
	275 280 285	
5	GAT AAT GAC CTT TTC CTT CGA ATG ACC TTT AAC GTG ATA AAC AAC ACA	912
	Asp Asn Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr	
	290 295 300	
10	AAT GGC GAA AAG ACG ATA CGT GGT TTG TTT CCA ACA CTG TAC CTC GGA	960
	Asn Gly Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly	
	305 310 315	
15	AAC TCT ACC GCT CTT GTT GCC CTC CTG AAC AAG GAT TTC CCT GAA TTA	1008
	Asn Ser Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu	
	320 325 330	
20	GGT GTA GAA ATT TCA GAT TGT ATT GAA ATG AGT TGG ATC GAG TCT GTT	1056
	Gly Val Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val	
	335 340 345 350	
	CTT TTC TAC ACA AAC TTC CCC ATT GGT ACT CCG ACC ACT GCT CTT CTA	1104
	Leu Phe Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu	
	355 360 365	
25	AGC CGT ACA CCT CAA AGA CTA AAC CCA TTC AAA ATC AAA TCT GAT TAC	1152
	Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr	
	370 375 380	
30	GTA AAA AAC ACT ATT TCC AAA CAG GGA TTC GAA TCC ATA TTT GAA AGG	1200
	Val Lys Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg	
	385 390 395	
35	ATG AAA GAA CTC GAA AAC CAA ATG CTA GCT TTC AAC CCT TAT GGT GGA	1248
	Met Lys Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly	
	400 405 410	
40	AGA ATG AGC GAA ATT TCC GAA TTT GCA AAG CCT TTT CCC CAT CGA TCA	1296
	Arg Met Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser	
	415 420 425 430	
	GGG AAT ATA GCG AAG ATC CAA TAC GAA GTA AAC TGG GAT GAA CTT GGC	1344
	Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly	
	435 440 445	
45	GTT GAA GCA GCC AAT CGG TAC TTG AAC TTC ACA AGG GTG ATG TAT GAT	1392
	Val Glu Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp	
	450 455 460	
50	TAT ATG ACT CCG TTT GTT TCT AAG AAC CCC AGG GAA GCA TTT CTG AAC	1440
	Tyr Met Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn	
	465 470 475	
55	TAC AGG GAT TTA GAT ATT GGT GTC AAC AGT CAT GGC AAG AAT GCT TAC	1488
	Tyr Arg Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr	
	480 485 490	

GGT GAA GGA ATG GTT TAT GGG CAC AAG TAT TTC AAA GAG ACG AAT TAT 1536  
 Gly Glu Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr  
 495 500 505 510

5 AAG AGG CTA ACG ATG GTG AAG ACG AGG GTT GAT CCT AGC AAT TTT TTT 1584  
 Lys Arg Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe  
 515 520 525

AGG AAT GAG CAA AGT ATC CCA ACT TTG TCA TCT TCA TGG AAG 1626  
 10 Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys  
 530 535 540

TAAATTCTAA ATTCACCTTGT GAAATTGAAT AAAAGTATGG CTTTTTCAAG GTCATGGTAT 1686

15 CCAGATTCAG ATGATATTGA TATAATTTTG ACTTGTATTT ATACAAACAA AATTATATTA 1746

TATTTTTCTG AATTTAGATT TTCCATTCTT TGGAAAAATA TACGAACATT GATGTTGATA 1806

TTTTTAAGAA TTATAGATTT TGAACATTGT GAACAATGAA TAAACCGAGG ACTTCCCTTG 1866

20 GGTTTTTTTT ATAAGTATGT AATAGCATGT CTTTAATCAA GATAACCGAT CATTGGATGC 1926

AATTTATTAT TATAAACCTT ATTTAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAA 1981

25 (2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 540 amino acids  
 (B) TYPE: amino acid  
 30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

35 Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe Pro Leu  
 1 5 10 15

Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr Ser Ile  
 40 20 25 30

Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro Ser Phe  
 35 40 45

45 Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn Ser Ser Phe Pro Ser  
 50 55 60

Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe Asn Glu Ser Thr Thr  
 65 70 75 80

50 Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His Pro Ser His Ile Gln  
 85 90 95

Ala Ala Val Val Cys Ala Lys Thr His Arg Leu Leu Met Lys Thr Arg  
 55 100 105 110

Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Val Thr Asn Ser Asn  
 115 120 125

5 Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu Arg Ser Ile Asn Val  
 130 135 140

Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu Gly  
 145 150 155 160

10 Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Ser His Ala Phe Pro  
 165 170 175

Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly Gly  
 15 180 185 190

Gly Tyr Gly Asn Leu Met Gly Lys Tyr Gly Leu Ser Val Asp Asn Ile  
 195 200 205

20 Val Asp Ala Gln Leu Ile Asp Val Asn Gly Lys Leu Leu Asn Arg Lys  
 210 215 220

Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly Val  
 225 230 235 240

25 Ser Phe Gly Val Val Val Ala Tyr Lys Ile Lys Leu Val Arg Val Pro  
 245 250 255

Thr Thr Val Thr Val Phe Asn Val Gln Arg Thr Ser Glu Gln Asn Leu  
 30 260 265 270

Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu Asp Asn  
 275 280 285

35 Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr Asn Gly  
 290 295 300

Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly Asn Ser  
 305 310 315 320

40 Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly Val  
 325 330 335

Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val Leu Phe  
 45 340 345 350

Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu Ser Arg  
 355 360 365

50 Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val Lys  
 370 375 380

Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg Met Lys  
 385 390 395 400

55

Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg Met  
 405 410 415  
 Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly Asn  
 5 420 425 430  
 Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly Val Glu  
 435 440 445  
 10 Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp Tyr Met  
 450 455 460  
 Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn Tyr Arg  
 465 470 475 480  
 15 Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr Gly Glu  
 485 490 495  
 Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys Arg  
 20 500 505 510  
 Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe Arg Asn  
 515 520 525  
 25 Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys  
 530 535 540

## (2) INFORMATION FOR SEQ ID NO: 59:

- 30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

- 35 (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GGTAATGATC TCCTTTCTTG TTTGACC

27

## (2) INFORMATION FOR SEQ ID NO: 60:

- 45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 41 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 50 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

55

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

5 AGAGCGGCCG CTATATTACA ACTTCTCCAC CATCACTCCT C 41

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

20 GGTGATGTTA ATGATAATCT CCTC 24

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

AGAGCGGCCG CTACAATTCC TTCAACATGT AAATTTTCCTC 40

(2) INFORMATION FOR SEQ ID NO: 63:

40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

55 ACTTCCCGTA GAAACTCGGA GACTTTCACA CAATGC 36

## (2) INFORMATION FOR SEQ ID NO: 64:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

10

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

15 TCCATCCAAG ATCAATTCAT AAAGTGTGTC

30

## (2) INFORMATION FOR SEQ ID NO: 65:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 25 (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

30

AGAGCGGCCG CTTTCATGAA CCTAGCTTCT AGTAGG

36

## (2) INFORMATION FOR SEQ ID NO: 66:

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 37 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

AGAGCGGCCG CGAAATGGCC CCCCTTTTAA AACGGGG

37

## (2) INFORMATION FOR SEQ ID NO: 67:

50

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

AGAGCGGCCG CAAATGATAT CTCAGGTAA CTTGTTCAC

40

(2) INFORMATION FOR SEQ ID NO: 68:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

AGAGCGGCCG CATAATCAAA TAAATACACT TATGGTAACA CAG

43

25 (2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

AGAGCGGCCG CTGGTTTTGT ATTGAGGACT CAAAACAG

38

40

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1757 base pairs

45

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

55



## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(B) STRAIN: Colombia

## 5 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: join(1..570, 801..1754)

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC CTA ACC TCA AAC 48  
 Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn  
 1 5 10 15  
 15 TCC GAC CCC AAA CAT CCC ATC TCC CCC GCT ATC TTC TTC TCC GGA AAT 96  
 Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn  
 20 25 30  
 20 GGC TCC TAC TCC TCC GTA TTA CAA GCC AAC ATC CGT AAC CTC CGC TTC 144  
 Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe  
 35 40 45  
 AAC ACC ACC TCA ACT CCG AAA CCC TTC CTC ATA ATC GCC GCA ACA CAT 192  
 25 Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His  
 50 55 60  
 GAA TCC CAT GTG CAA GCC GCG ATT ACT TGC GGG AAA CGC CAC AAC CTT 240  
 30 Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu  
 65 70 75 80  
 CAG ATG AAA ATC AGA AGT GGA GGC CAC GAC TAC GAT GGC TTG TCA TAC 288  
 Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr  
 85 90 95  
 35 GTT ACA TAC TCT GGC AAA CCG TTC TTC GTC CTC GAC ATG TTT AAC CTC 336  
 Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu  
 100 105 110  
 40 CGT TCG GTG GAT GTC GAT GTG GCA AGT AAG ACC GCG TGG GTC CAA ACC 384  
 Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr  
 115 120 125  
 GGT GCC ATA CTC GGA GAA GTT TAT TAC TAT ATA TGG GAG AAG AGC AAA 432  
 45 Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys  
 130 135 140  
 ACC CTA GCT TAT CCC GCC GGA ATT TGT CCC ACG GTT GGT GTC GGT GGC 480  
 Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly  
 50 145 150 155 160  
 CAT ATC AGT GGT GGA GGT TAC GGT AAC ATG ATG AGA AAA TAC GGT CTC 528  
 His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu  
 165 170 175

ACC GTA GAT AAT ACC ATC GAT GCA AGA ATG GTC GAC GTT AAT 570  
 Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn  
 180 185 190

5 GGTATAATTG ATATCTCTAT TTTATATACT AATTAAATTT TATAGTGTGG ATCGGATAGT 630  
 GATTTTGGTC CATCAATTAA AAACCTGGTG AACATAAAAT TAACCAAGCA ATCAATTTAG 690  
 ACAAGCAACA TAATCATATA TATTTTCTT ACATTTGTAT GTACCTGAAT ATTTATATTT 750

10 ATGTTTATAT GTTCTCACTA TATTTTCACT TTTGTATTTG AAAATTTTTA GGA AAA 806  
 Gly Lys

ATT TTG GAT AGA AAA TTG ATG GGA GAA GAT CTC TAC TGG GCA ATA AAC 854  
 15 Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn  
 195 200 205

GGA GGA GGA GGA GGG AGC TAC GGC GTC GTA TTG GCC TAC AAA ATA AAC 902  
 Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn  
 20 210 215 220

CTT GTT GAA GTC CCA GAA AAC GTC ACC GTT TTC AGA ATC TCC CGG ACG 950  
 Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr  
 225 230 235 240

25 TTA GAA CAA AAT GCG ACG GAT ATC ATT CAC CGG TGG CAA CAA GTT GCA 998  
 Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala  
 245 250 255

30 CCG AAG CTT CCC GAC GAG CTT TTC ATA AGA ACA GTC ATT GAC GTA GTA 1046  
 Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val  
 260 265 270

AAC GGC ACT GTT TCA TCT CAA AAG ACC GTC AGG ACA ACA TTC ATA GCA 1094  
 35 Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala  
 275 280 285

ATG TTT CTA GGA GAC ACG ACA ACT CTA CTG TCG ATA TTA AAC CGG AGA 1142  
 40 Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg  
 290 295 300

TTC CCA GAA TTG GGT TTG GTC CGG TCT GAC TGT ACC GAA ACA AGC TGG 1190  
 Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp  
 305 310 315 320

45 ATC CAA TCT GTG CTA TTC TGG ACA AAT ATC CAA GTT GGT TCG TCG GAG 1238  
 Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu  
 325 330 335

50 ACA CTT CTA CTC CAA AGG AAT CAA CCC GTG AAC TAC CTC AAG AGG AAA 1286  
 Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys  
 340 345 350

55

TCA GAT TAC GTA CGT GAA CCG ATT TCA AGA ACC GGT TTA GAG TCA ATT 1334  
 Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile  
 355 360 365

5 TGG AAG AAA ATG ATC GAG CTT GAA ATT CCG ACA ATG GCT TTC AAT CCA 1382  
 Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro  
 370 375 380

10 TAC GGT GGT GAG ATG GGG AGG ATA TCA TTA CGG GTG ACT CCG TTC CCA 1430  
 Tyr Gly Gly Glu Met Gly Arg Ile Ser Leu Arg Val Thr Pro Phe Pro  
 385 390 395 400

15 TAC AGA GCC GGT AAT CTC TGG AAG ATT CAG TAC GGT GCG AAT TGG AGA 1478  
 Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg  
 405 410 415

GAT GAG ACT TTA ACC GAC CGG TAC ATG GAA TTG ACG AGG AAG TTG TAC 1526  
 Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr  
 420 425 430

20 CAA TTC ATG ACA CCA TTT GTT TCC AAG AAT CCG AGA CAA TCG TTT TTC 1574  
 Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe  
 435 440 445

25 AAT AAC CGT GAT GTT GAT TTG GGT ATT AAT TCT CAT AAT GGT AAA ATC 1622  
 Asn Asn Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile  
 450 455 460

30 AGT AGT TAT GTG GAA GGT AAA CGT TAC GGG AAG AAG TAT TTC GCA GGT 1670  
 Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly  
 465 470 475 480

35 AAT TTC GAG AGA TTG GTG AAG ATT AAG ACG AGA GTT GAT AGT GGT AAT 1718  
 Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn  
 485 490 495

TTC TTT AGG AAC GAA CAC AGT ATT CCT GTG TTA CCA TAA 1757  
 Phe Phe Arg Asn Glu His Ser Ile Pro Val Leu Pro  
 500 505

40

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 508 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn  
 1 5 10 15

55

Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn  
 20 25 30  
 Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe  
 5 35 40 45  
 Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His  
 50 55 60  
 10 Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu  
 65 70 75 80  
 Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr  
 85 90 95  
 15 Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu  
 100 105 110  
 Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr  
 115 120 125  
 Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys  
 130 135 140  
 25 Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly  
 145 150 155 160  
 His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu  
 165 170 175  
 30 Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn Gly Lys  
 180 185 190  
 Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn  
 195 200 205  
 Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn  
 210 215 220  
 40 Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr  
 225 230 235 240  
 Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala  
 245 250 255  
 45 Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val  
 260 265 270  
 Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala  
 275 280 285  
 50 Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg  
 290 295 300  
 55

Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp  
 305 310 315 320  
 5 Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu  
 325 330 335  
 Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys  
 340 345 350  
 10 Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile  
 355 360 365  
 Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro  
 370 375 380  
 15 Tyr Gly Gly Glu Met Gly Arg Ile Ser Leu Arg Val Thr Pro Phe Pro  
 385 390 395 400  
 Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg  
 20 405 410 415  
 Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr  
 420 425 430  
 25 Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe  
 435 440 445  
 Asn Asn Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile  
 450 455 460  
 30 Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly  
 465 470 475 480  
 Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn  
 35 485 490 495  
 Phe Phe Arg Asn Glu His Ser Ile Pro Val Leu Pro  
 500 505

40 (2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1527 base pairs  
 (B) TYPE: nucleic acid  
 45 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

50 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

55

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*  
 (B) STRAIN: Colombia

## 5 (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1524

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC CTA ACC TCA AAC 48  
 Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn  
 1 5 10 15

15 TCC GAC CCC AAA CAT CCC ATC TCC CCC GCT ATC TTC TTC TCC GGA AAT 96  
 Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn  
 20 25 30

20 GGC TCC TAC TCC TCC GTA TTA CAA GCC AAC ATC CGT AAC CTC CGC TTC 144  
 Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe  
 35 40 45

AAC ACC ACC TCA ACT CCG AAA CCC TTC CTC ATA ATC GCC GCA ACA CAT 192  
 25 Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His  
 50 55 60

GAA TCC CAT GTG CAA GCC GCG ATT ACT TGC GGG AAA CGC CAC AAC CTT 240  
 Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu  
 30 65 70 75 80

CAG ATG AAA ATC AGA AGT GGA GGC CAC GAC TAC GAT GGC TTG TCA TAC 288  
 Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr  
 85 90 95

35 GTT ACA TAC TCT GGC AAA CCG TTC TTC GTC CTC GAC ATG TTT AAC CTC 336  
 Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu  
 100 105 110

40 CGT TCG GTG GAT GTC GAC GTG GCA AGT AAG ACC GCG TGG GTC CAA ACC 384  
 Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr  
 115 120 125

GGT GCC ATA CTC GGA GAA GTT TAT TAC TAT ATA TGG GAG AAG AGC AAA 432  
 45 Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys  
 130 135 140

ACC CTA GCT TAT CCC GCC GGA ATT TGT CCC ACG GTT GGT GTC GGT GGC 480  
 Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly  
 50 145 150 155 160

CAT ATC AGT GGT GGA GGT TAC GGT AAC ATG ATG AGA AAA TAC GGT CTC 528  
 His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu  
 165 170 175

55

	ACC	GTA	GAT	AAT	ACC	ATC	GAT	GCA	AGA	ATG	GTC	GAC	GTA	AAT	GGA	AAA	576
	Thr	Val	Asp	Asn	Thr	Ile	Asp	Ala	Arg	Met	Val	Asp	Val	Asn	Gly	Lys	
				180					185						190		
5	ATT	TTG	GAT	AGA	AAA	TTG	ATG	GGA	GAA	GAT	CTC	TAC	TGG	GCA	ATA	AAC	624
	Ile	Leu	Asp	Arg	Lys	Leu	Met	Gly	Glu	Asp	Leu	Tyr	Trp	Ala	Ile	Asn	
			195					200					205				
	GGA	GGA	GGA	GGA	GGG	AGC	TAC	GGC	GTC	GTA	TTG	GCC	TAC	AAA	ATA	AAC	672
10	Gly	Gly	Gly	Gly	Gly	Ser	Tyr	Gly	Val	Val	Leu	Ala	Tyr	Lys	Ile	Asn	
		210						215					220				
	CTT	GTT	GAA	GTC	CCA	GAA	AAC	GTC	ACC	GTT	TTC	AGA	ATC	TCC	CGG	ACG	720
	Leu	Val	Glu	Val	Pro	Glu	Asn	Val	Thr	Val	Phe	Arg	Ile	Ser	Arg	Thr	
15	225					230					235					240	
	TTA	GAA	CAA	AAT	GCG	ACG	GAT	ATC	ATT	CAC	CGG	TGG	CAA	CAA	GTT	GCA	768
	Leu	Glu	Gln	Asn	Ala	Thr	Asp	Ile	Ile	His	Arg	Trp	Gln	Gln	Val	Ala	
					245					250					255		
20	CCG	AAG	CTT	CCC	GAC	GAG	CTT	TTC	ATA	AGA	ACA	GTC	ATT	GAC	GTA	GTA	816
	Pro	Lys	Leu	Pro	Asp	Glu	Leu	Phe	Ile	Arg	Thr	Val	Ile	Asp	Val	Val	
				260						265				270			
25	AAC	GGC	ACT	GTT	TCA	TCT	CAA	AAG	ACC	GTC	AGG	ACA	ACA	TTC	ATA	GCA	864
	Asn	Gly	Thr	Val	Ser	Ser	Gln	Lys	Thr	Val	Arg	Thr	Thr	Phe	Ile	Ala	
			275					280					285				
	ATG	TTT	CTA	GGA	GAC	ACG	ACA	ACT	CTA	CTG	TCG	ATA	TTA	AAC	CGG	AGA	912
30	Met	Phe	Leu	Gly	Asp	Thr	Thr	Thr	Leu	Leu	Ser	Ile	Leu	Asn	Arg	Arg	
		290						295					300				
	TTC	CCA	GAA	TTG	GGT	TTG	GTC	CGG	TCT	GAC	TGT	ACC	GAA	ACA	AGC	TGG	960
	Phe	Pro	Glu	Leu	Gly	Leu	Val	Arg	Ser	Asp	Cys	Thr	Glu	Thr	Ser	Trp	
35	305					310					315					320	
	ATC	CAA	TCT	GTG	CTA	TTC	TGG	ACA	AAT	ATC	CAA	GTT	GGT	TCG	TCG	GAG	1008
	Ile	Gln	Ser	Val	Leu	Phe	Trp	Thr	Asn	Ile	Gln	Val	Gly	Ser	Ser	Glu	
					325					330					335		
40	ACA	CTT	CTA	CTC	CAA	AGG	AAT	CAA	CCC	GTG	AAC	TAC	CTC	AAG	AGG	AAA	1056
	Thr	Leu	Leu	Leu	Gln	Arg	Asn	Gln	Pro	Val	Asn	Tyr	Leu	Lys	Arg	Lys	
				340						345				350			
45	TCA	GAT	TAC	GTA	CGT	GAA	CCG	ATT	TCA	AGA	ACC	GGT	TTA	GAG	TCA	ATT	1104
	Ser	Asp	Tyr	Val	Arg	Glu	Pro	Ile	Ser	Arg	Thr	Gly	Leu	Glu	Ser	Ile	
			355					360					365				
	TGG	AAG	AAA	ATG	ATC	GAG	CTT	GAA	ATT	CCG	ACA	ATG	GCT	TTC	AAT	CCA	1152
50	Trp	Lys	Lys	Met	Ile	Glu	Leu	Glu	Ile	Pro	Thr	Met	Ala	Phe	Asn	Pro	
		370						375					380				
	TAC	GGT	GGT	GAG	ATG	GGG	AGG	ATA	TCA	TCT	ACG	GTG	ACT	CCG	TTC	CCA	1200
	Tyr	Gly	Gly	Glu	Met	Gly	Arg	Ile	Ser	Ser	Thr	Val	Thr	Pro	Phe	Pro	
55	385						390				395					400	

TAC AGA GCC GGT AAT CTC TGG AAG ATT CAG TAC GGT GCG AAT TGG AGA 1248  
 Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg  
 405 410 415

5 GAT GAG ACT TTA ACC GAC CGG TAC ATG GAA TTG ACG AGG AAG TTG TAC 1296  
 Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr  
 420 425 430

10 CAA TTC ATG ACA CCA TTT GTT TCC AAG AAT CCG AGA CAA TCG TTT TTC 1344  
 Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe  
 435 440 445

15 AAT TAC CGT GAT GTT GAT TTG GGT ATT AAT TCT CAT AAT GGT AAA ATC 1392  
 Asn Tyr Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile  
 450 455 460

20 AGT AGT TAT GTG GAA GGT AAA CGT TAC GGG AAG AAG TAT TTC GCA GGT 1440  
 Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly  
 465 470 475 480

AAT TTC GAG AGA TTG GTG AAG ATT AAG ACG AGA GTT GAT AGT GGT AAT 1488  
 Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn  
 485 490 495

25 TTC TTT AGG AAC GAA CAG AGT ATT CCT GTG TTA CCA TAA 1527  
 Phe Phe Arg Asn Glu Gln Ser Ile Pro Val Leu Pro  
 500 505

30 (2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 508 amino acids  
 (B) TYPE: amino acid  
 35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

40 Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn  
 1 5 10 15

Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn  
 45 20 25 30

Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe  
 35 40 45

50 Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His  
 50 55 60

Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu  
 65 70 75 80

55



[illegible]

Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro  
 370 375 380  
 Tyr Gly Gly Glu Met Gly Arg Ile Ser Ser Thr Val Thr Pro Phe Pro  
 5 385 390 395 400  
 Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg  
 405 410 415  
 10 Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr  
 420 425 430  
 Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe  
 435 440 445  
 15 Asn Tyr Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile  
 450 455 460  
 Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly  
 20 465 470 475 480  
 Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn  
 485 490 495  
 25 Phe Phe Arg Asn Glu Gln Ser Ile Pro Val Leu Pro  
 500 505

## (2) INFORMATION FOR SEQ ID NO: 74:

- 30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1530 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear  
 35 (ii) MOLECULE TYPE: cDNA to mRNA  
 (iii) HYPOTHETICAL: NO  
 40 (iii) ANTI-SENSE: NO  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Arabidopsis thaliana  
 (B) STRAIN: Colombia  
 45 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1527  
 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

TCC ATC CAA GAT CAA TTC ATA AAC TGT GTC AAA AGA AAC ACA CAT GTT 48  
 Ser Ile Gln Asp Gln Phe Ile Asn Cys Val Lys Arg Asn Thr His Val  
 55 1 5 10 15

	TCT	TTT	CCA	CTC	GAG	AAA	ACG	TTA	TTC	ACC	CCT	GCG	AAA	AAC	GTC	TCT	96
	Ser	Phe	Pro	Leu	Glu	Lys	Thr	Leu	Phe	Thr	Pro	Ala	Lys	Asn	Val	Ser	
				20					25						30		
5	TTG	TTC	AAC	CAA	GTC	CTT	GAA	TCG	ACG	GCT	CAA	AAT	CTC	CAG	TTC	TTG	144
	Leu	Phe	Asn	Gln	Val	Leu	Glu	Ser	Thr	Ala	Gln	Asn	Leu	Gln	Phe	Leu	
			35					40					45				
	GCA	AAA	TCC	ATG	CCT	AAA	CCG	GGA	TTC	ATA	TTC	AGA	CCG	ATT	CAC	CAG	192
10	Ala	Lys	Ser	Met	Pro	Lys	Pro	Gly	Phe	Ile	Phe	Arg	Pro	Ile	His	Gln	
		50					55					60					
	TCT	CAA	GTC	CAA	GCT	TCC	ATC	ATT	TGT	TCA	AAG	AAA	CTC	GGA	ATT	CAT	240
	Ser	Gln	Val	Gln	Ala	Ser	Ile	Ile	Cys	Ser	Lys	Lys	Leu	Gly	Ile	His	
15	65					70				75					80		
	TTT	CGT	GTT	AGA	AGT	GGC	GGT	CAC	GAT	TTC	GAG	GCC	TTG	TCT	TAT	GTT	288
	Phe	Arg	Val	Arg	Ser	Gly	Gly	His	Asp	Phe	Glu	Ala	Leu	Ser	Tyr	Val	
				85					90						95		
20	TCA	CGG	ATT	GAA	AAA	CCG	TTT	ATA	TTA	CTC	GAC	CTG	TCA	AAA	TTG	AAA	336
	Ser	Arg	Ile	Glu	Lys	Pro	Phe	Ile	Leu	Leu	Asp	Leu	Ser	Lys	Leu	Lys	
				100				105					110				
25	CAA	ATC	AAT	GTT	GAT	ATT	GAA	TCC	AAT	AGT	GCT	TGG	GTT	CAA	CCT	GGT	384
	Gln	Ile	Asn	Val	Asp	Ile	Glu	Ser	Asn	Ser	Ala	Trp	Val	Gln	Pro	Gly	
			115					120					125				
	GCT	ACG	CTT	GGT	GAG	CTT	TAC	TAC	AGA	ATT	GCA	GAG	AAG	AGC	AAG	ATC	432
30	Ala	Thr	Leu	Gly	Glu	Leu	Tyr	Tyr	Arg	Ile	Ala	Glu	Lys	Ser	Lys	Ile	
		130					135					140					
	CAT	GGA	TTT	CCC	GCG	GGT	TTG	TGC	ACA	AGT	GTA	GGC	ATA	GGT	GGG	TAT	480
	His	Gly	Phe	Pro	Ala	Gly	Leu	Cys	Thr	Ser	Val	Gly	Ile	Gly	Gly	Tyr	
35	145					150				155					160		
	ATG	ACA	GGC	GGT	GGA	TAC	GGT	ACC	TTG	ATG	AGG	AAG	TAT	GGT	CTT	GCG	528
	Met	Thr	Gly	Gly	Gly	Tyr	Gly	Thr	Leu	Met	Arg	Lys	Tyr	Gly	Leu	Ala	
				165				170					175				
40	GGA	GAT	AAT	GTT	CTA	GAC	GTA	AAG	ATG	GTT	GAT	GCA	AAT	GGT	AAA	TTA	576
	Gly	Asp	Asn	Val	Leu	Asp	Val	Lys	Met	Val	Asp	Ala	Asn	Gly	Lys	Leu	
				180				185					190				
45	CTC	GAC	AGA	GCC	GCG	ATG	GGT	GAG	GAC	CTA	TTT	TGG	GCG	ATT	AGA	GGA	624
	Leu	Asp	Arg	Ala	Ala	Met	Gly	Glu	Asp	Leu	Phe	Trp	Ala	Ile	Arg	Gly	
			195					200					205				
	GGC	GGT	GGA	GCG	AGT	TTC	GGG	ATA	GTT	CTA	GCA	TGG	AAG	ATC	AAG	CTT	672
50	Gly	Gly	Gly	Ala	Ser	Phe	Gly	Ile	Val	Leu	Ala	Trp	Lys	Ile	Lys	Leu	
		210					215					220					
	GTT	CCT	GTT	CCT	AAG	ACT	GTT	ACC	GTC	TTC	ACT	GTC	ACC	AAA	ACG	TTA	720
	Val	Pro	Val	Pro	Lys	Thr	Val	Thr	Val	Phe	Thr	Val	Thr	Lys	Thr	Leu	
55	225					230				235					240		

	GAA CAA GAC GCA AGA TTG AAG ACT ATT TCT AAG TGG CAA CAA ATT TCA	768
	Glu Gln Asp Ala Arg Leu Lys Thr Ile Ser Lys Trp Gln Gln Ile Ser	
	245 250 255	
5	TCC AAG ATT ATT GAA GAG ATA CAC ATC CGA GTG GTA CTC AGA GCA GCT	816
	Ser Lys Ile Ile Glu Glu Ile His Ile Arg Val Val Leu Arg Ala Ala	
	260 265 270	
10	GGA AAT GAT GGA AAC AAG ACT GTG ACA ATG ACC TAC CTA GGT CAG TTT	864
	Gly Asn Asp Gly Asn Lys Thr Val Thr Met Thr Tyr Leu Gly Gln Phe	
	275 280 285	
15	CTT GGC GAG AAA GGC ACC TTG CTG AAG GTT ATG GAG AAG GCT TTT CCA	912
	Leu Gly Glu Lys Gly Thr Leu Leu Lys Val Met Glu Lys Ala Phe Pro	
	290 295 300	
20	GAA CTA GGG TTA ACT CAA AAG GAT TGT ACT GAA ATG AGC TGG ATT GAA	960
	Glu Leu Gly Leu Thr Gln Lys Asp Cys Thr Glu Met Ser Trp Ile Glu	
	305 310 315 320	
25	GCC GCC CTT TTC CAT GGT GGA TTT CCA ACA GGT TCT CCT ATT GAA ATT	1008
	Ala Ala Leu Phe His Gly Gly Phe Pro Thr Gly Ser Pro Ile Glu Ile	
	325 330 335	
30	TTG CTT CAG CTC AAG TCG CCT CTA GGA AAA GAT TAC TTC AAA GCA ACG	1056
	Leu Leu Gln Leu Lys Ser Pro Leu Gly Lys Asp Tyr Phe Lys Ala Thr	
	340 345 350	
35	TCG GAT TTC GTT AAA GAA CCT ATT CCT GTG ATA GGC TTC AAA GGA ATA	1104
	Ser Asp Phe Val Lys Glu Pro Ile Pro Val Ile Gly Phe Lys Gly Ile	
	355 360 365	
40	TTC AAA AGA TTG ATT GAA GGA AAC ACA ACA TTT CTG AAC TGG ACT CCT	1152
	Phe Lys Arg Leu Ile Glu Gly Asn Thr Thr Phe Leu Asn Trp Thr Pro	
	370 375 380	
45	TAC GGT GGT ATG ATG TCG AAA ATC CCT GAA TCT GCG ATC CCA TTT CCG	1200
	Tyr Gly Gly Met Met Ser Lys Ile Pro Glu Ser Ala Ile Pro Phe Pro	
	385 390 395 400	
50	CAT AGA AAC GGA ACC CTC TTC AAG ATT CTC TAT TAC GCG AAC TGG CTA	1248
	His Arg Asn Gly Thr Leu Phe Lys Ile Leu Tyr Tyr Ala Asn Trp Leu	
	405 410 415	
55	GAG AAT GAC AAG ACA TCG AGT AGA AAA ATC AAC TGG ATC AAA GAG ATA	1296
	Glu Asn Asp Lys Thr Ser Ser Arg Lys Ile Asn Trp Ile Lys Glu Ile	
	420 425 430	
60	TAC AAT TAC ATG GCG CCT TAT GTC TCA AGC AAT CCA AGA CAA GCA TAT	1344
	Tyr Asn Tyr Met Ala Pro Tyr Val Ser Ser Asn Pro Arg Gln Ala Tyr	
	435 440 445	
65	GTG AAC TAC AGA GAT CTA GAC TTC GGA CAG AAC AAG AAC AAC GCA AAG	1392
	Val Asn Tyr Arg Asp Leu Asp Phe Gly Gln Asn Lys Asn Asn Ala Lys	
	450 455 460	

GTT AAC TTC ATT GAA GCT AAA ATC TGG GGA CCT AAG TAC TTC AAA GGC 1440  
 Val Asn Phe Ile Glu Ala Lys Ile Trp Gly Pro Lys Tyr Phe Lys Gly  
 465 470 475 480

5 AAT TTT GAC AGA TTG GTG AAG ATT AAA ACC AAG GTT GAT CCA GAG AAC 1488  
 Asn Phe Asp Arg Leu Val Lys Ile Lys Thr Lys Val Asp Pro Glu Asn  
 485 490 495

TTC TTC AGG CAC GAG CAG AGT ATC CCA CCT ATG CCC TAC TAG 1530  
 10 Phe Phe Arg His Glu Gln Ser Ile Pro Pro Met Pro Tyr  
 500 505

## (2) INFORMATION FOR SEQ ID NO: 75:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 509 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

Ser Ile Gln Asp Gln Phe Ile Asn Cys Val Lys Arg Asn Thr His Val  
 25 1 5 10 15

Ser Phe Pro Leu Glu Lys Thr Leu Phe Thr Pro Ala Lys Asn Val Ser  
 20 25 30

Leu Phe Asn Gln Val Leu Glu Ser Thr Ala Gln Asn Leu Gln Phe Leu  
 30 35 40 45

Ala Lys Ser Met Pro Lys Pro Gly Phe Ile Phe Arg Pro Ile His Gln  
 50 55 60

Ser Gln Val Gln Ala Ser Ile Ile Cys Ser Lys Lys Leu Gly Ile His  
 35 65 70 75 80

Phe Arg Val Arg Ser Gly Gly His Asp Phe Glu Ala Leu Ser Tyr Val  
 40 85 90 95

Ser Arg Ile Glu Lys Pro Phe Ile Leu Leu Asp Leu Ser Lys Leu Lys  
 100 105 110

Gln Ile Asn Val Asp Ile Glu Ser Asn Ser Ala Trp Val Gln Pro Gly  
 45 115 120 125

Ala Thr Leu Gly Glu Leu Tyr Tyr Arg Ile Ala Glu Lys Ser Lys Ile  
 130 135 140

His Gly Phe Pro Ala Gly Leu Cys Thr Ser Val Gly Ile Gly Gly Tyr  
 50 145 150 155 160

Met Thr Gly Gly Gly Tyr Gly Thr Leu Met Arg Lys Tyr Gly Leu Ala  
 55 165 170 175

Gly Asp Asn Val Leu Asp Val Lys Met Val Asp Ala Asn Gly Lys Leu  
 — 180 185 190

5 Leu Asp Arg Ala Ala Met Gly Glu Asp Leu Phe Trp Ala Ile Arg Gly  
 195 200 205

Gly Gly Gly Ala Ser Phe Gly Ile Val Leu Ala Trp Lys Ile Lys Leu  
 210 215 220

10 Val Pro Val Pro Lys Thr Val Thr Val Phe Thr Val Thr Lys Thr Leu  
 225 230 235 240

Glu Gln Asp Ala Arg Leu Lys Thr Ile Ser Lys Trp Gln Gln Ile Ser  
 15 245 250 255

Ser Lys Ile Ile Glu Glu Ile His Ile Arg Val Val Leu Arg Ala Ala  
 260 265 270

20 Gly Asn Asp Gly Asn Lys Thr Val Thr Met Thr Tyr Leu Gly Gln Phe  
 275 280 285

Leu Gly Glu Lys Gly Thr Leu Leu Lys Val Met Glu Lys Ala Phe Pro  
 290 295 300

25 Glu Leu Gly Leu Thr Gln Lys Asp Cys Thr Glu Met Ser Trp Ile Glu  
 305 310 315 320

Ala Ala Leu Phe His Gly Gly Phe Pro Thr Gly Ser Pro Ile Glu Ile  
 30 325 330 335

Leu Leu Gln Leu Lys Ser Pro Leu Gly Lys Asp Tyr Phe Lys Ala Thr  
 340 345 350

35 Ser Asp Phe Val Lys Glu Pro Ile Pro Val Ile Gly Phe Lys Gly Ile  
 355 360 365

Phe Lys Arg Leu Ile Glu Gly Asn Thr Thr Phe Leu Asn Trp Thr Pro  
 370 375 380

40 Tyr Gly Gly Met Met Ser Lys Ile Pro Glu Ser Ala Ile Pro Phe Pro  
 385 390 395 400

His Arg Asn Gly Thr Leu Phe Lys Ile Leu Tyr Tyr Ala Asn Trp Leu  
 45 405 410 415

Glu Asn Asp Lys Thr Ser Ser Arg Lys Ile Asn Trp Ile Lys Glu Ile  
 420 425 430

50 Tyr Asn Tyr Met Ala Pro Tyr Val Ser Ser Asn Pro Arg Gln Ala Tyr  
 435 440 445

Val Asn Tyr Arg Asp Leu Asp Phe Gly Gln Asn Lys Asn Asn Ala Lys  
 450 455 460

55

Val Asn Phe Ile Glu Ala Lys Ile Trp Gly Pro Lys Tyr Phe Lys Gly  
465 470 475 480

Asn Phe Asp Arg Leu Val Lys Ile Lys Thr Lys Val Asp Pro Glu Asn  
5                   485                         490                             495

Phe Phe Arg His Glu Gln Ser Ile Pro Pro Met Pro Tyr  
500 505

## AMENDED CLAIMS

1. An isolated protein which has antifungal activity, preferably anti-Oomycete activity, and more preferably anti-*Phytophthora* and/or anti-*Pythium* activity and which is obtainable from a plant source, encoded by the nucleotide sequence as shown in SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 57, SEQ ID NO: 70, SEQ ID NO: 72 or SEQ ID NO: 74 or parts or muteins thereof.
2. An isolated protein according to claim 1, characterised in that the protein is naturally occurring in sunflower or lettuce.
3. An isolated plant protein having carbohydrate oxidase activity, characterised in that it has antifungal activity, preferably anti-Oomycete activity, and more preferably anti-*Phytophthora* and/or anti-*Pythium* activity.
4. A carbohydrate oxidase, characterised in that it has an amino acid sequence according to SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 58, SEQ ID NO: 71, SEQ ID NO: 73 or SEQ ID NO: 75 or parts or muteins therefrom.
5. An isolated antifungal protein, characterised in that it comprises one or more of the peptides selected from the group consisting of:
  - (a) amino acids 1 to 25 of SEQ ID NO: 1,
  - (b) amino acids 1 to 25 of SEQ ID NO: 2,
  - (c) amino acids 1 to 118 of SEQ ID NO: 6,
  - (d) amino acids 1 to 529 of SEQ ID NO 16, or a part of said sequence having antifungal activity,
  - (e) amino acids 1 to 529 of SEQ ID NO 20, or a part of said sequence having antifungal activity,
  - (f) amino acids 1 to 21 of SEQ ID NO: 49
  - (g) amino acids 1 to 24 of SEQ ID NO: 50
  - (h) amino acids 1 to 14 of SEQ ID NO: 51
  - (i) amino acids 1 to 540 of SEQ ID NO 58, or a part of said sequence having antifungal activity,
  - (j) amino acids 1 to 508 of SEQ ID NO 71, or a part of said sequence having antifungal activity,
  - (k) amino acids 1 to 508 of SEQ ID NO 73, or a part of said sequence having antifungal activity,
  - (l) amino acids 1 to 509 of SEQ ID NO 75, or a part of said sequence having antifungal activity,as well as muteins thereof which have antifungal activity.
6. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 15, or by part of said open reading frame.



7. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 57, or by part of said open reading frame.
8. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 70, or by part of said open reading frame.
9. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 72, or by part of said open reading frame.
10. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 74, or by part of said open reading frame.
11. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 19, or by part of said open reading frame.
12. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by one of the open reading frames represented by SEQ ID NO's: 21 - 48.
13. An isolated DNA sequence comprising an open reading frame capable of encoding a protein according to any of the claims 1 to 12, and DNA capable of hybridising therewith under stringent conditions.
14. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 5.
15. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 15.
16. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 19.
17. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 21-48.

18. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 57.
19. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 70.
20. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 72.
21. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 74.
22. A chimeric DNA sequence comprising a DNA sequence according to any of claims 13 to 21.
23. A chimeric DNA sequence according to claim 22, further comprising a transcriptional initiation region and, optionally, a transcriptional termination region, linked to the open reading frame which is capable of expressing a protein of any of claims 1-12.
24. A chimeric DNA sequence according to claim 23, wherein the RNA comprising said open reading frame is capable of being translated into protein in said host cell, when present therein, thereby producing said protein.
25. A chimeric DNA sequence according to any one of claims 22 to 24 which is a replicon.
26. A chimeric DNA sequence according to claim 25 which is a vector.
27. A vector according to claim 26, which is a binary vector.
28. A host cell comprising a replicon according to claim 25 and which is capable of maintaining said replicon once present therein.
29. A host cell comprising a vector according to claim 26 or 27 and which is capable of maintaining said vector once present therein.
30. A host cell stably incorporating in its genome a chimeric DNA sequence according to claim 22 or 23.
31. A host cell according to claim 30 which is a plant cell, said vector being a non-integrative viral vector.

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32. A host cell according to claim 30 which is a plant cell.
33. A plant or a plant part comprising at least one plant cell according to claim 31 or 32.
34. A plant or a plant part consisting essentially of plant cells according to claim 32.
35. A plant according to claim 34, characterised in that said chimeric DNA is expressed in at least a number of the plant's cells causing the said antifungal protein to be produced therein.
36. A method for the production of a protein with antifungal activity, preferably anti-Oomycete activity, and more preferably anti-*Phytophthora* and/or anti-*Pythium* activity, characterised in that a host cell according to claim 29 to 32 is grown under favourable conditions and that said protein is recovered from the host cells.
37. A method for the production of a protein with antifungal activity, preferably anti-Oomycete activity, and more preferably anti-*Phytophthora* and/or anti-*Pythium* activity, characterised in that a host cell according to claim 29 to 32 is grown under favourable conditions and that said protein is isolated from the culture medium.
38. Use of a protein according to any one of claims 1 to 12 for retarding fungal growth, preferably Oomycete growth and more preferably the growth of *Phytophthora sp.* and/or *Pythium sp.*
39. A plant-derived protein having a molecular weight of 55-65 kD, characterized in that it has carbohydrate oxidising activity.
40. A protein according to claim 39, characterized in that it is a hexose oxidase.
41. A protein according to claim 40, characterized in that it is obtainable from sunflower or lettuce plants.
42. A method of retarding the growth of the a fungus, preferably an Oomycete, more preferably *Phytophthora* or *Pythium* on plant leaves, characterised in that the plant is treated with a protein produced from a host cell according to claim 29 or 30, or from a cell of a plant according to claim 35.

43. A method for obtaining plants with reduced susceptibility to fungi, preferably Oomycetes, more preferably *Phytophthora* or *Pythium*, comprising the steps of
- (a) introducing into ancestor cells which are susceptible of regeneration into a whole plant,
    - a chimeric DNA sequence comprising an open reading frame capable of encoding a protein according to any of claims 1-12, said open reading frame being operatively linked to a transcriptional and translational region and, optionally, a transcriptional termination region, allowing the said protein to be produced in a plant cell that is susceptible to infection by said fungus and
    - a chimeric DNA sequence capable of encoding a plant selectable marker allowing selection of transformed ancestor cells when said selectable marker is present therein, and
  - (b) regenerating said ancestor cells into a plant under conditions favouring ancestor cells which have the said selectable marker, and
  - (c) identifying a plant which produces a protein according to claim 1-7, thereby reducing the susceptibility of said plant to infection by said fungus.
44. The method according to claim 43, characterised in that step (a) is performed using an *Agrobacterium tumefaciens* strain capable of T-DNA transfer to plant cells and which harbours a binary vector, and wherein step (b) is performed in the presence of an antibiotic favouring cells which have a neomycin phosphotransferase.
45. An antifungal composition comprising a protein according to any one of claims 1 to 12, and a suitable carrier.
46. An antibody capable of recognising a protein according to any one of claims 1 to 12.
47. A nucleic acid sequence obtainable from a gene encoding a protein according to any one of claims 1 to 12, having tissue-specific and/or developmental specific transcriptional regulatory activity in a plant.
48. A nucleic acid sequence according to claim 47, which is obtainable from the region upstream of the translational initiation site of said gene.
49. A nucleic acid sequence according to claim 48, which has at least 1000 nucleotides of said region upstream of the translational initiation site of said gene.
50. Use of a nucleic acid sequence according to any one of claims 47 to 49 for making a plant expressible gene construct.

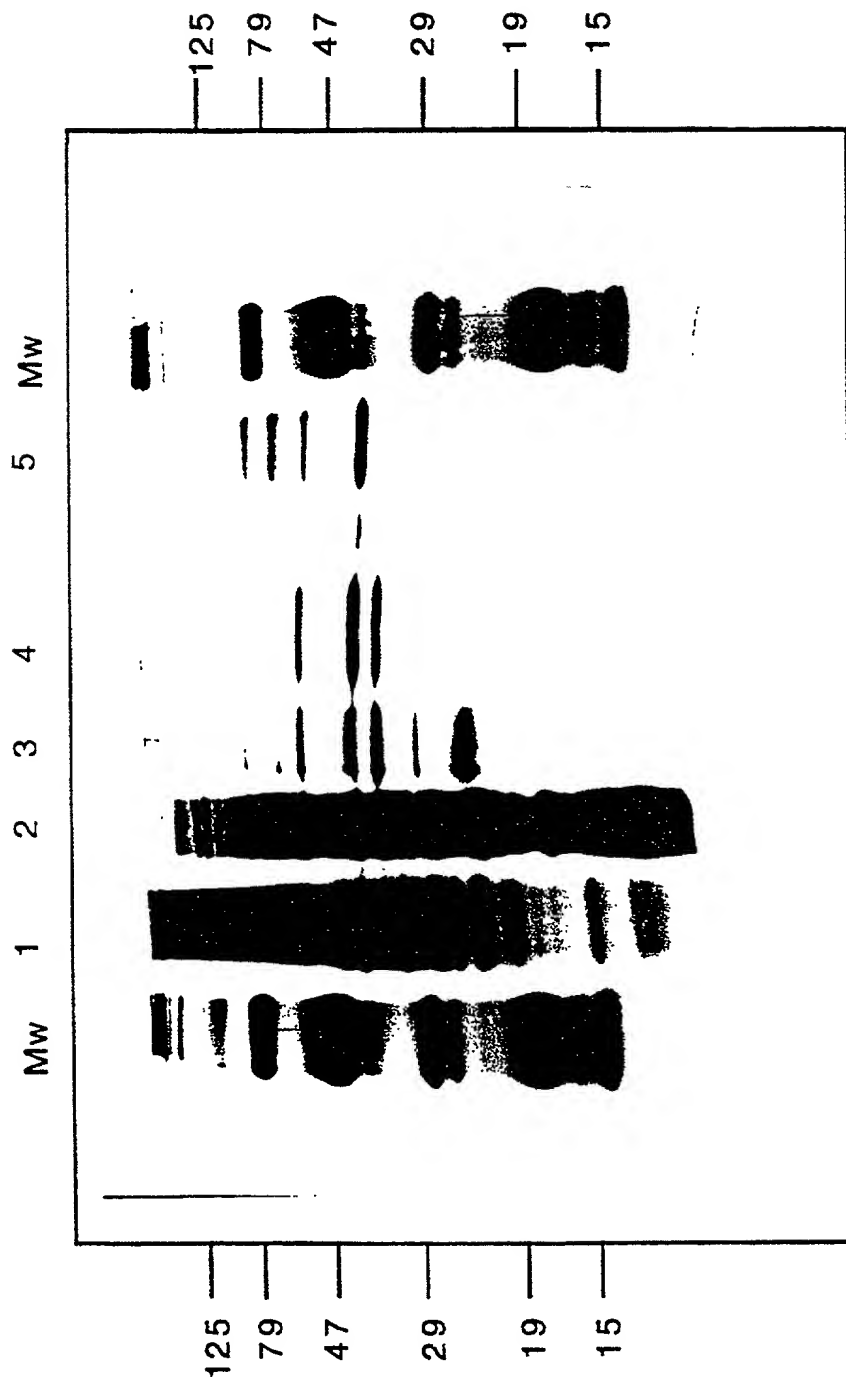


Fig. 1

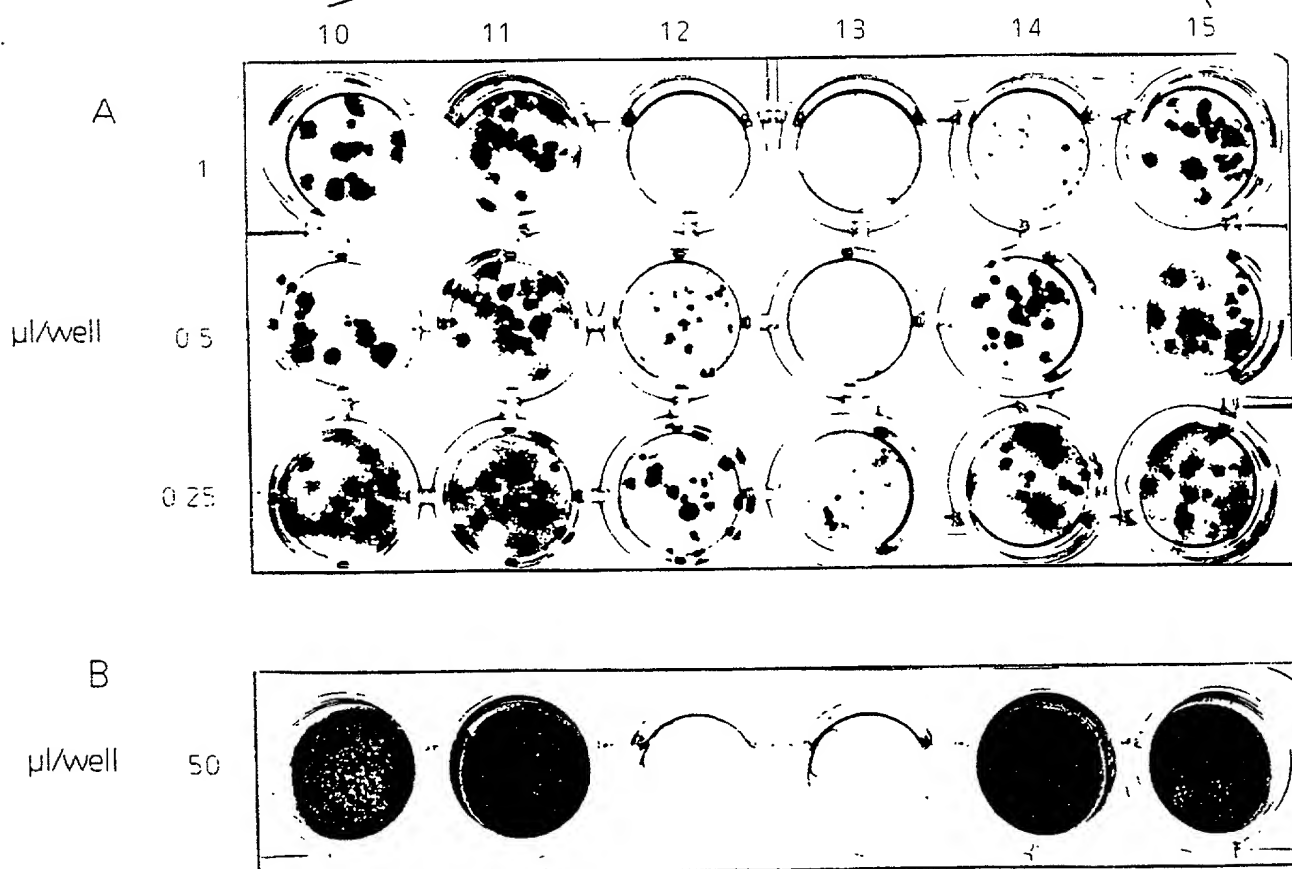
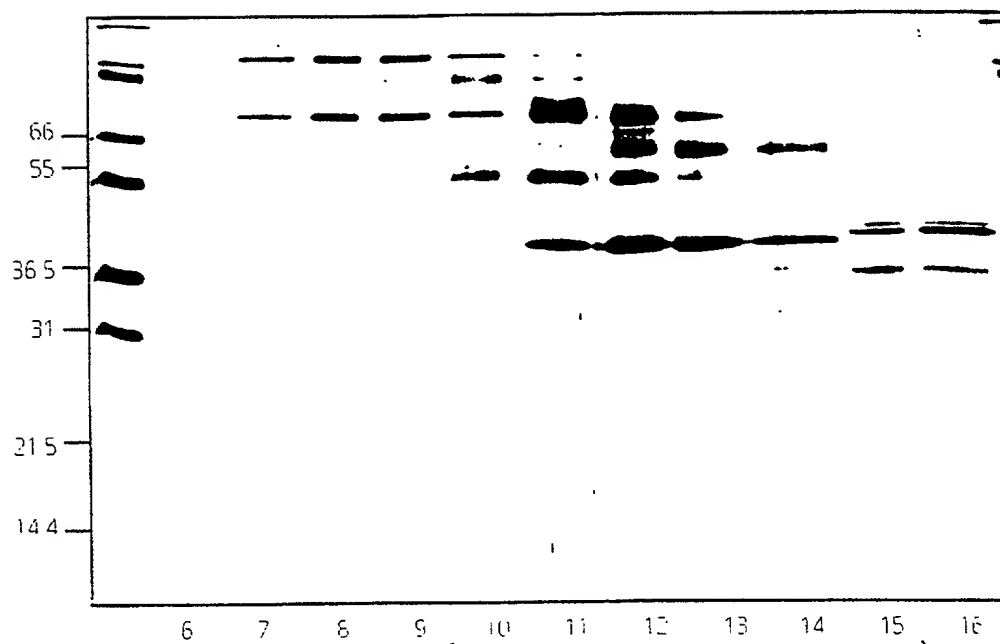
INVENTOR: M. H. **STUIVER**

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INVENTOR: M. H. **STUIVER**

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SHEET 2 OF 12

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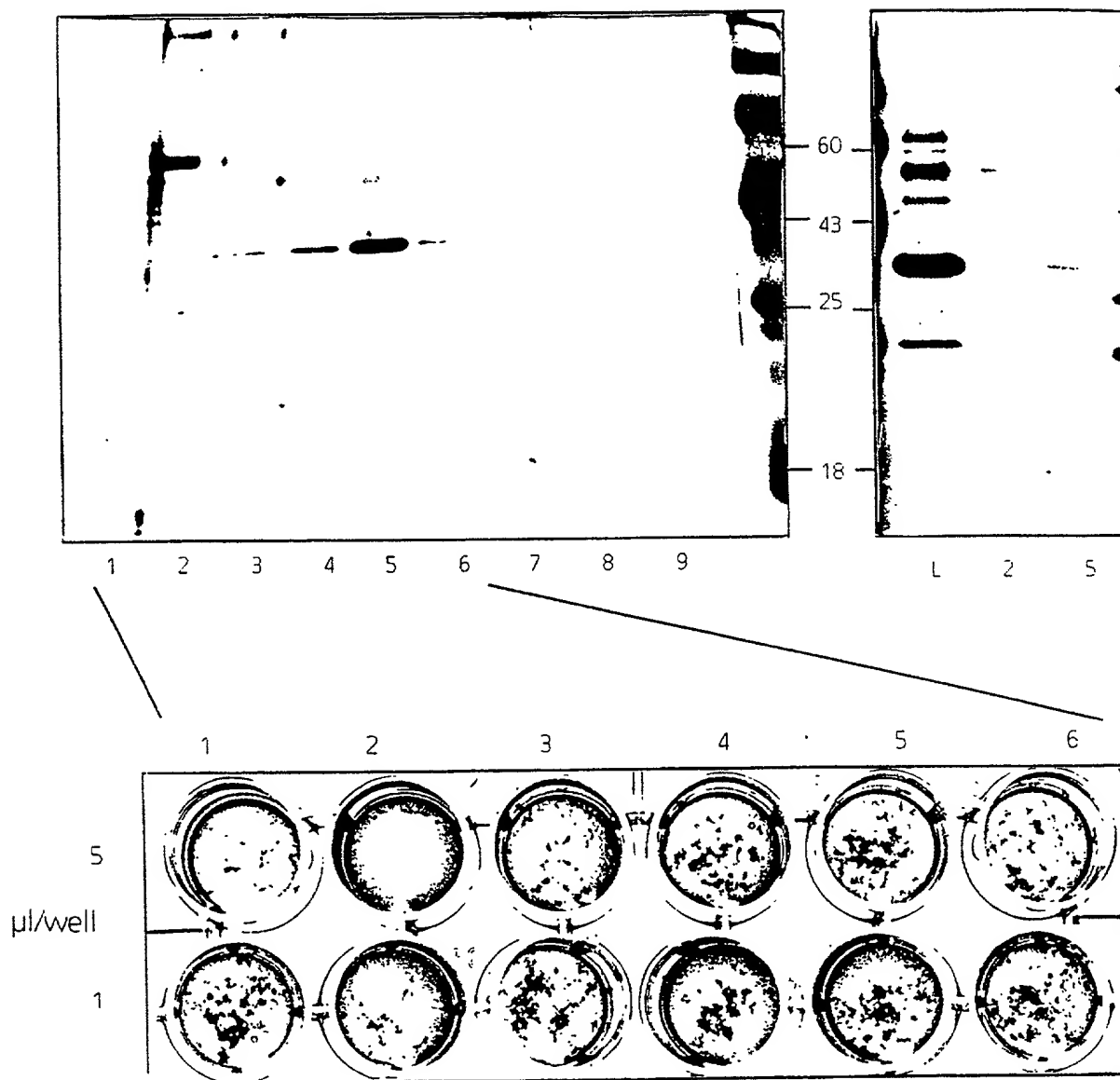


Fig. 3

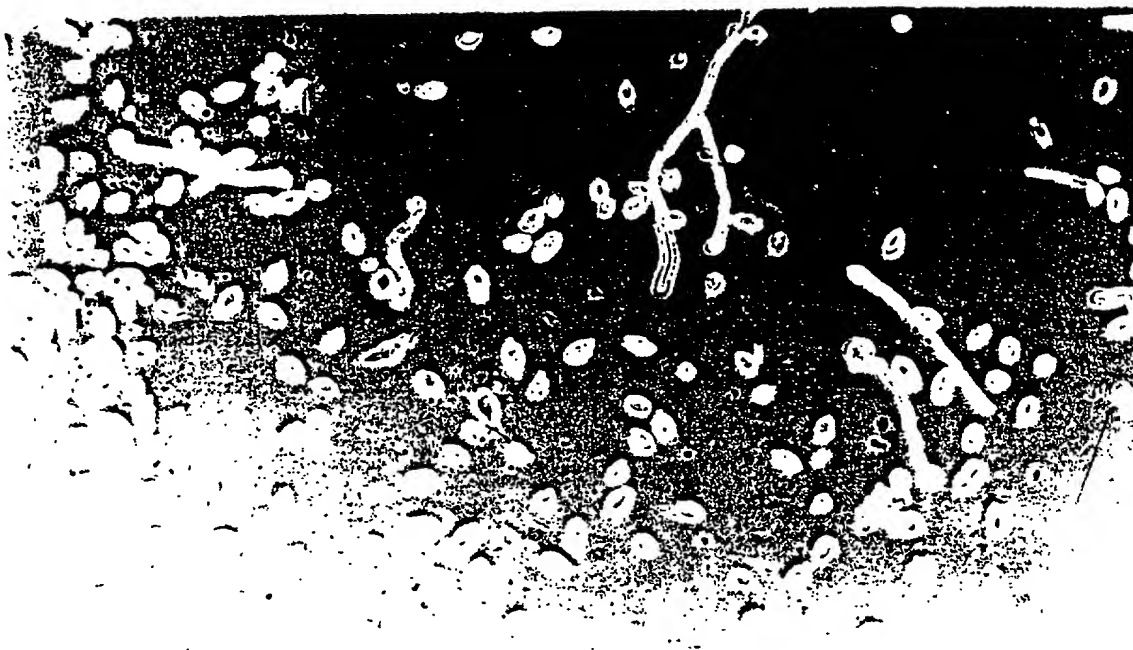
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INVENTOR: M. H. **STUIVER**

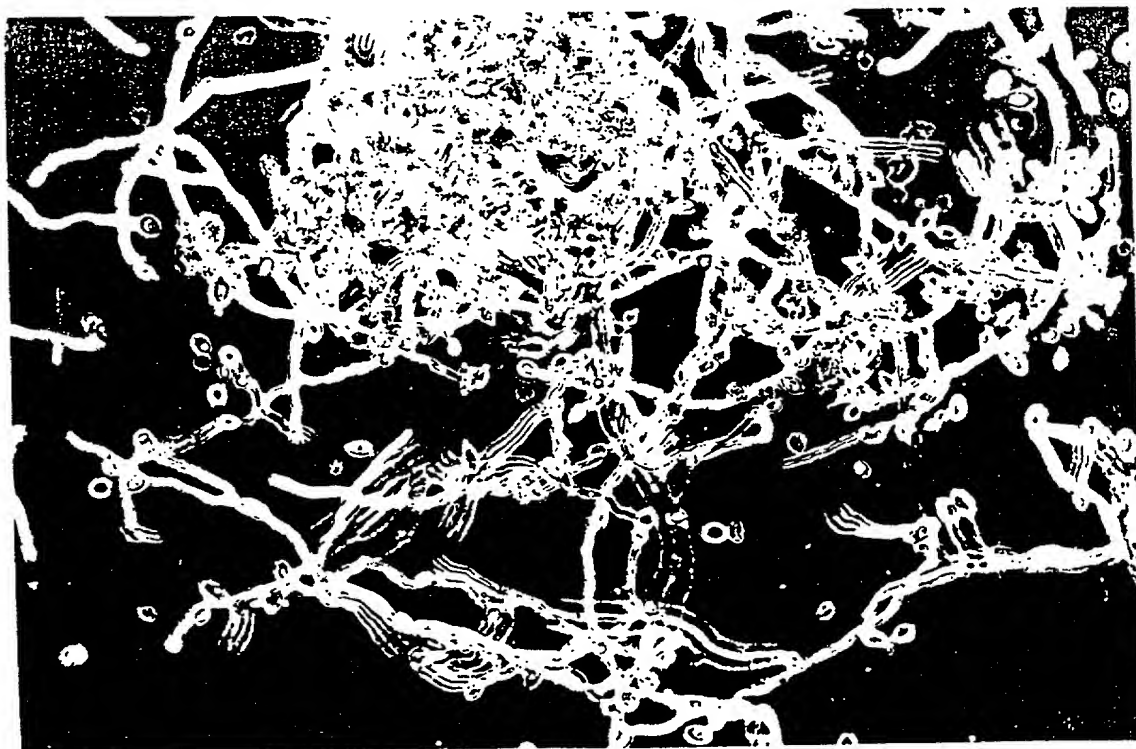
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SHEET 3 OF 12

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RIGHT



LEFT

Fig. 4

69 69 20 1 69 69 69

INVENTOR: M. H. **STUIVER**

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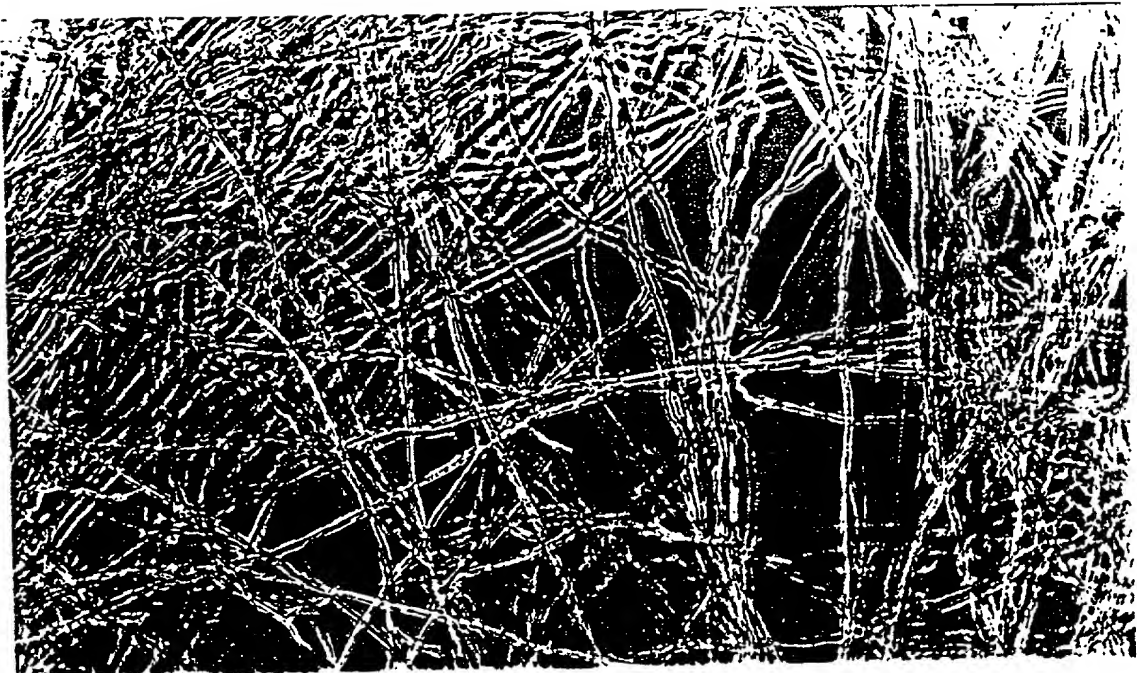
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665220" TE085260



RIGHT



LEFT

Fig. 5

INVENTOR: M. H. STUIVER

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6 A

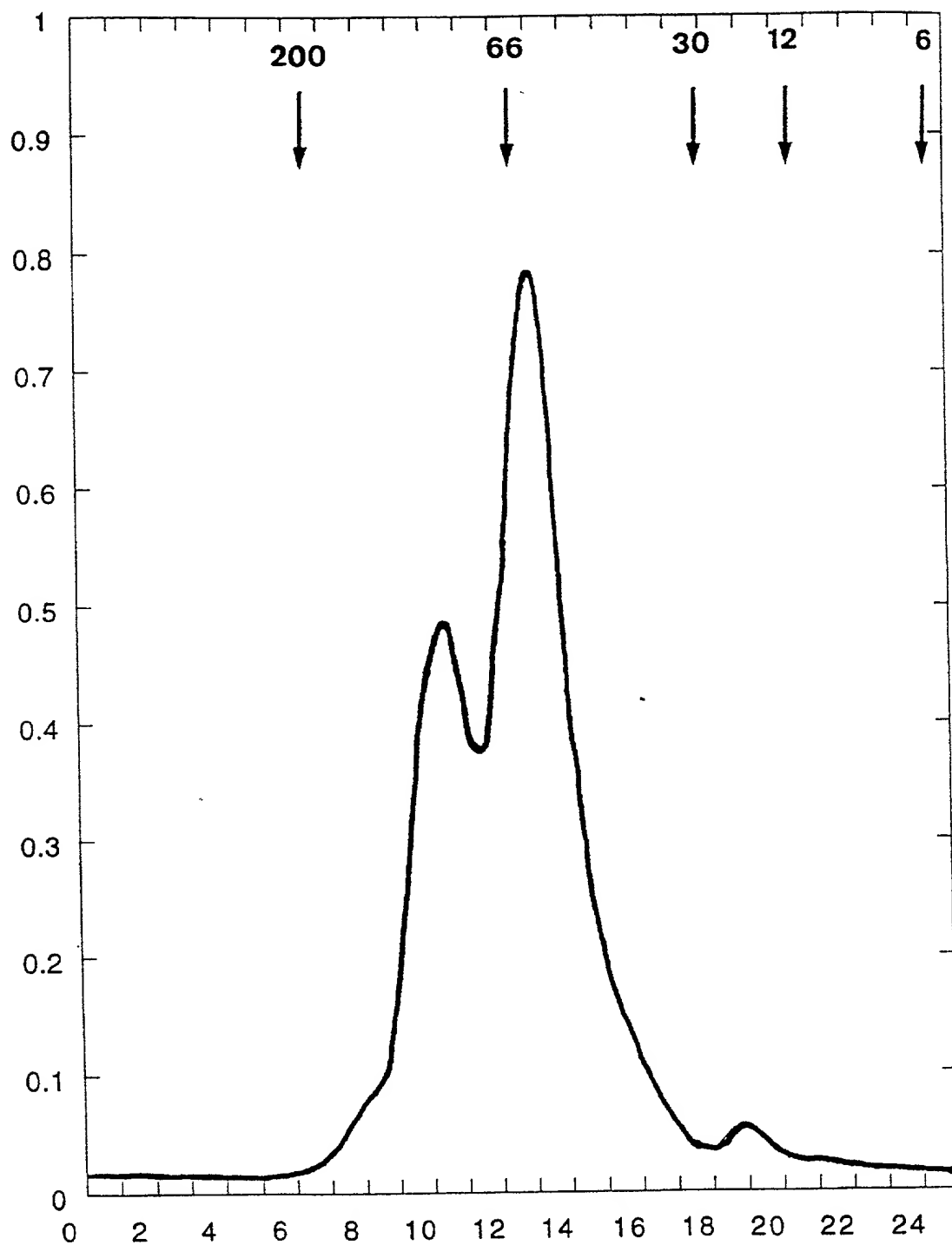


Fig. 6-1

665220-1609260

INVENTOR: M. H. **STUIVER**

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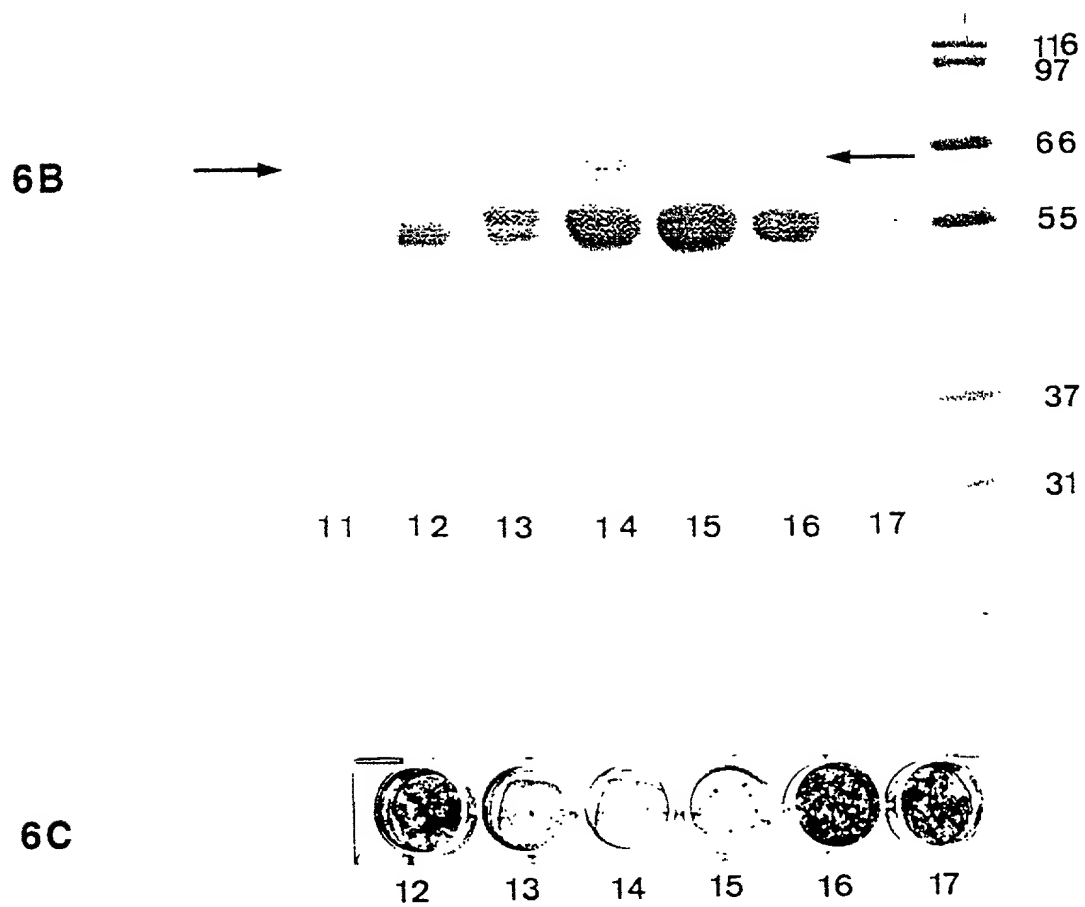


Fig. 6-2

66320-729260

INVENTOR: M. H. **STUIVER**

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NO.: EJ405329117US

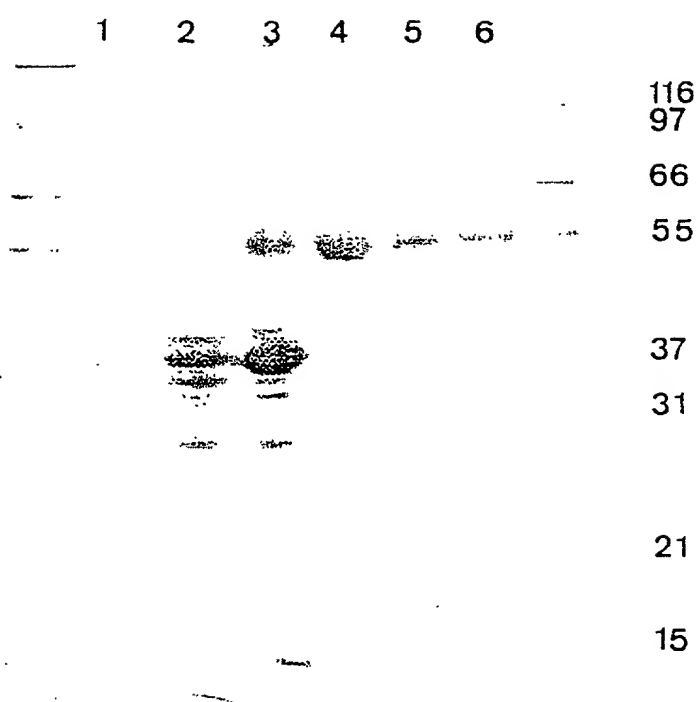


Fig. 7

Sheet 8 of 12

INVENTOR: M. H. **STUIVER**

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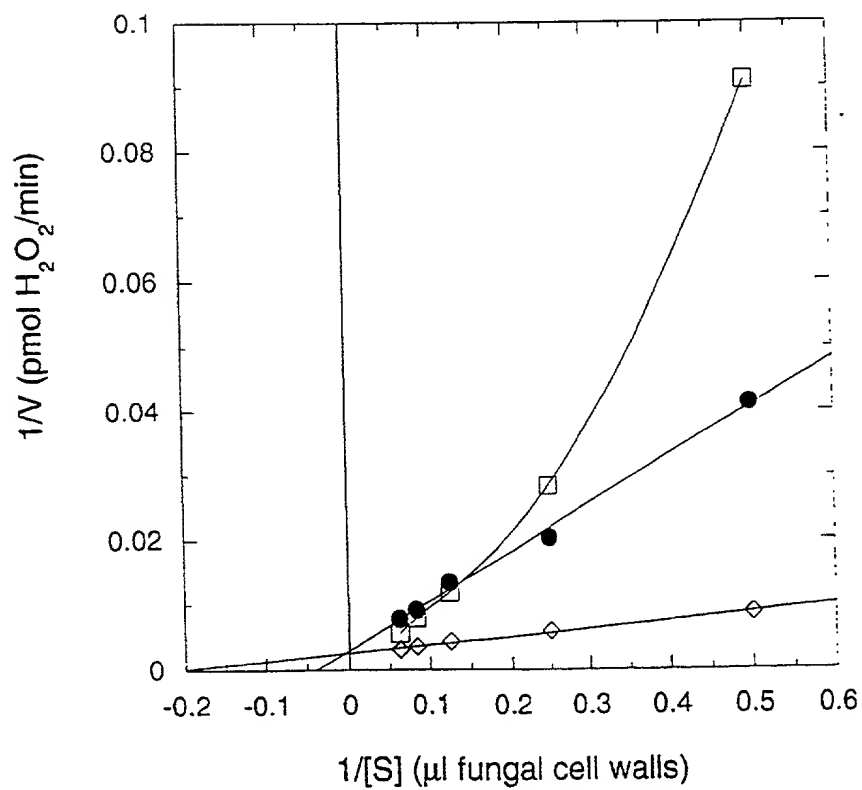
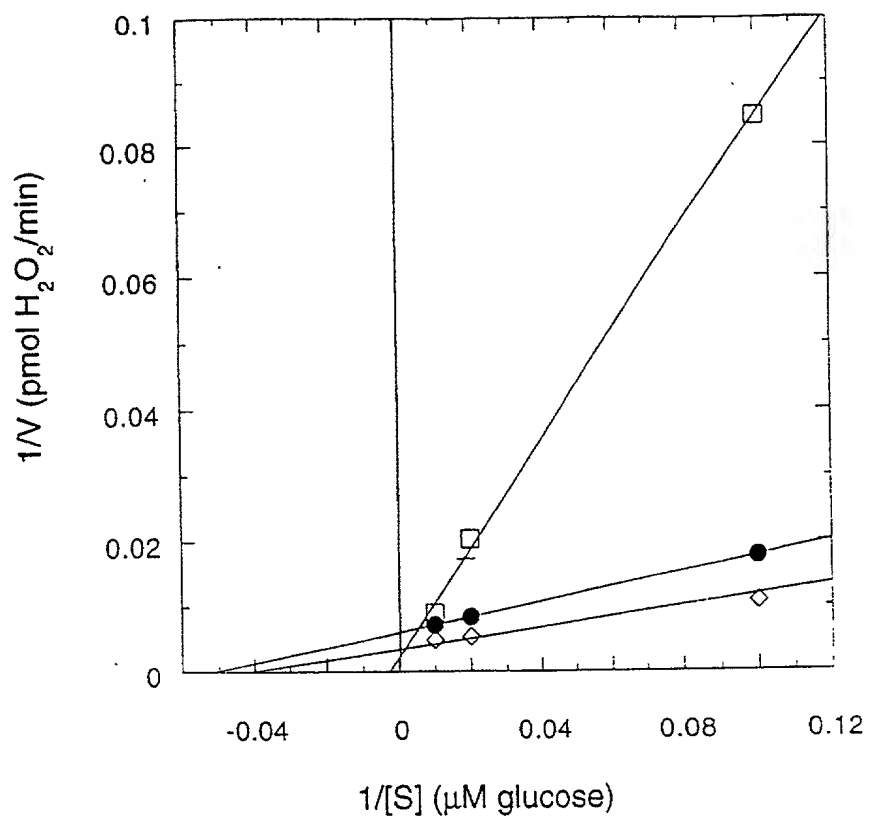


Fig. 8

65320" TCS25A

INVENTOR: M. H. **STUIVER**

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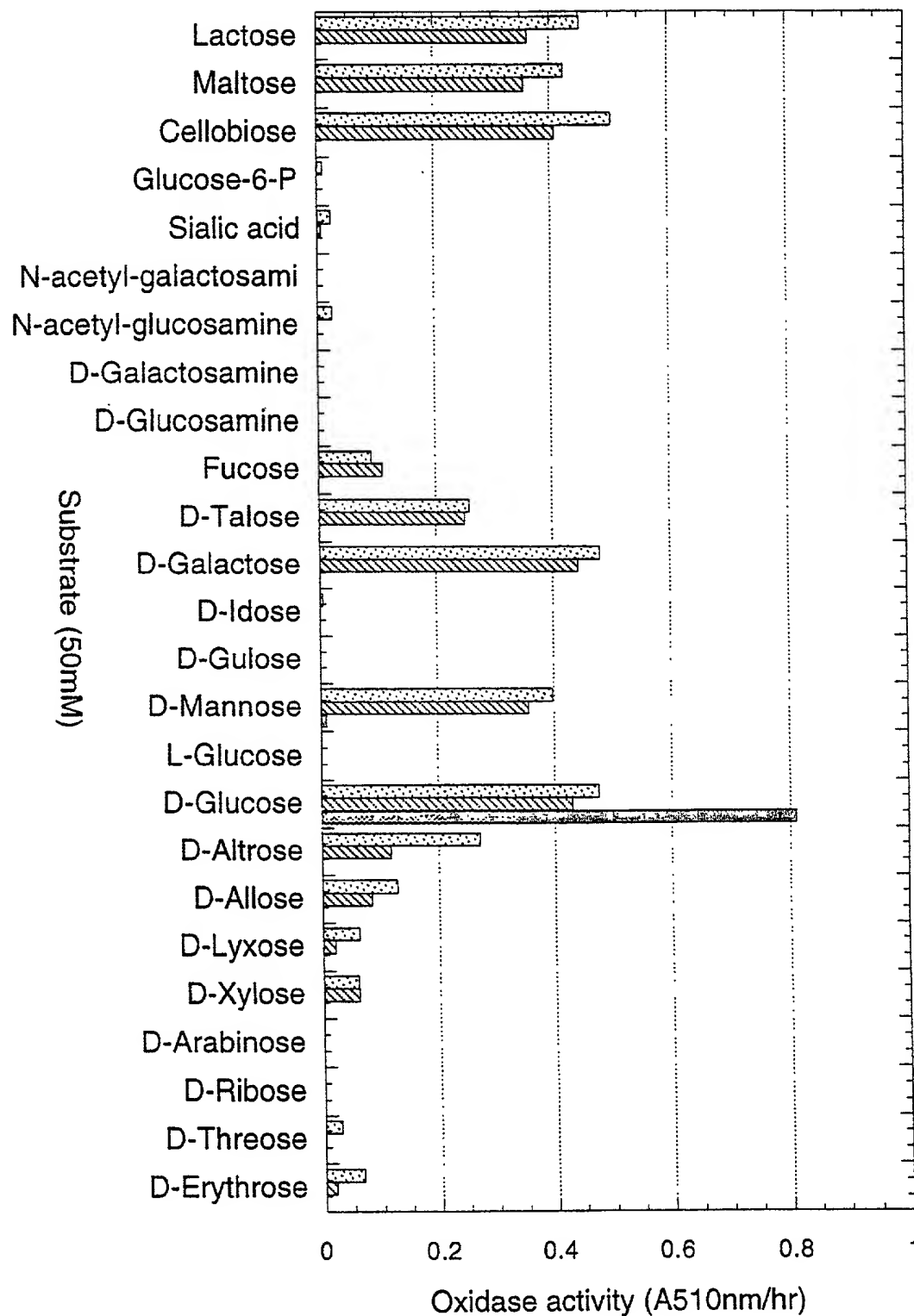


Fig. 9

663310-1000000

INVENTOR: M. H. **STUIVER**

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MS59 1 -----MQTS ILTL LLLLS TQSS SATSRSID-RFIQCLH DRADPSFP  
 WL64 1 MAITYSFNFKSY IFPL LVL LLS THSS SATSTSIID-RFTQCLNNRADPSFP  
 At26 1 -----TSRRNSE-TFTQCLT SNSDPKHP  
 At27 1 -----SIQD-QFINCVKRNTHVSFP  
 EcBBE 1 -----MENKTP IFFSL SIFLS L LNCALGG--ND--LLSCLTFNGVRNH-  
 PsBBE 1 -----MMCRSLT LRFFL FIVL-LQTCVRGGDVNDNLLSSCLNSHG VHNE-

MS59 42 ITGEVYTPGN--SSFP TVLQNYIRNLRFNETTTPKPFLLIITA EHVSHIQ A  
 WL64 50 LSGQLYTPDN--SSFPSVLQAYIRNLRFNESTTPKPII IITALHP SHIQ A  
 At26 23 ISPAIFFSGN--GSYSSVLQANIRNLRFNTTSTPKPFLLIITAATHESHVQ A  
 At27 20 LEKTLFTPAKNV SLENQVLESTAQN LQFLAKSMKPGFIFRPIHQSQVQ A  
 EcBBE 40 --TVFSADSD--SDENRFLHLSIQNPLFQNSLISKPSAILLP GSKEELSN  
 PsBBE 44 --TTLSWDTN--SDYFKLLHASM ONPLFAKPTVSKPSFHVMPGSKEELSS

MS59 90 AVVCGKQNRLL KTRSGGHDYEGLS YLTNTNQ PFFIVDMFNLR SINVDIE  
 WL64 98 AVVCAKTHRLLM KTRSGGHDYEGLS YVTNSNQ PFFIVDMFNLR SINVSIE  
 At26 71 AITCGKRHNLOM KIRSGGHDYDGLSYVTYSGK PFFVLD MFNLRSDVDVA  
 At27 70 SIICSKKLG IHRVRSGGHDYEGLS YVSRIEK PFIILLDL SKLQINVDIE  
 EcBBE 86 TIRCI RKGSWTI RLRSGGHSYEGLS YTSDT--PFIILLDL MNLNRV SIDIE  
 PsBBE 90 TVHCTRESWTI RLRSGGHSYEGLS YTADT--PEVIVDM MNLNRISIDVL

MS59 140 QETAWVQAGATLGEVYYRIA EKS NKHGFPAGV CPTVGVGGHFSGGGYGNL  
 WL64 148 DETAWVQAGATLGEVYYRIA EKS NSHAFAPAGV CPTVGVGGHFSGGGYGNL  
 At26 121 SKTAWVQTGAILGEVYYIWEKSKT LAYPAGI CPTVGVGGHISGGGYGNM  
 At27 120 SNSAWVQPGATLGEVYYRIA ESKIHGFPAGL CTSVGTG GYMTGGGYGTL  
 EcBBE 134 SETAWVESGSTLGEVYYAITESSSK LGFTAGW CPTVGTG GHISGGGF GMM  
 PsBBE 138 SETAWVESGATLGEVYYAIAQSTDT LGFTAGW CPTVGS GGHISGGGF GMM

MS59 190 MRKYGLSVDNIVDAQ IIDVNGKL LDRKSMGEDLFWAITGGGGVSFGVVLA  
 WL64 198 MGKYGLSVDNIVDAQ LIDVNGKL LNRKSMGEDLFWAITGGGGVSFGVVVA  
 At26 171 MRKYGLTVDNITIDARMVDVNGKILDRKLMGEDLFWAINGGGGSGYVVLA  
 At27 170 MRKYGLAGDNVLDVKMVDANGKLLDRAAMGEDLFWAIRGGGGVSGYVLA  
 EcBBE 184 SRKYGLAADNVVDAILIDANGA I LDRQAMGEDVFWAIRGGGGVWGAIYA  
 PsBBE 188 SRKYGLAADNVVDAILIDSNGA I LDREKMGDDVFWAIRGGGGVWGAIYA

MS59 240 YKIKLVRVPEVTVFTIERREEQNLS-TIAERWVQVADKLDRLD FLRMTF  
 WL64 248 YKIKLVRVPTTVTVFNVQRTSEQNLS-TIAHRWVQVADKLDNLD FLRMTF  
 At26 221 YKINLVEVPENVTVFRISRTLEQNAT-DIHRWQVAPKLPDELFI RTVI  
 At27 220 WKIKLVPVPKTVTVFTVTKTLEQDARLKTISKWQOISSKIIIEIHIRVVL  
 EcBBE 234 WKIKLLPVPEKVTVFRVTKNVAIDEATSL LHKWQFVAEELEEDF---TL  
 PsBBE 238 WKIKLLPVPEKLTVFRVTKNVGIEDASSL LHKWQYVADELEEDF---TV

MS59 289 SVINDTNG-GKTVRAIFPTLYLGNSRN LVTL LNKFEP ELGLQESDCTEMS  
 WL64 297 NVINNTNG-EKTI RGLFPTLYLGNS TALVAL LNKFEP ELGVEISDCIEMS  
 At26 270 DVVNGTVSSQKTVRTTEIAMFLGDTTLLSILNRRFEP ELGLVRSDCETES  
 At27 270 RAAGNDGN--KTVTMTYLGQFLGKGTLLKVMEKAFPELGLTQKDC TEMS  
 EcBBE 280 SVLGCAD--KQVWLTMLGFHFLGLKTVAKSTFDLLEPELGLVEEDY LEMS  
 PsBBE 284 SVLGGVNG--NDAWLMFGLHHLGRKDAAKTIIDEKEPELGLVDKEFQEMS

Fig. 10-1

66440-10000000

INVENTOR: M. H. **STUIVER**

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12/12

MS59 338 WVESVLYYTGFPSGTPTTALLS RTP - QRLNPFKIKSDYVQNPISKQFEEF  
 WL64 346 WIESVLFYTNFPIGTPTTALLS RTP - QRLNPFKIKSDYVKNITISKQGFES  
 At26 320 WIQSVLFWTNIQVGSSETLLQRN - QPVNYLKRKSDYVREPIISRTGLES  
 At27 318 WIEAALFHGGFPTGSSEIEILLQQLKSPLGKDYFKATSDFVKEPIPVIGFKG  
 EcBBE 328 WGESFAYLAGLET - - - VSQ LNNRFLKFDERAFKTKVDLTKEPLPSKAFYG  
 PsBBE 332 WGESMAFLSGLDT - - - ISELNNRFLKFDERAFKTKVDFTKVSVPLNVFRH

MS59 1 FIFERLKELENQMLAFNPYGGRMSEISEFAKPFPHRSGNIAKIQYEVNWE  
 WL64 1 SIFERMKELENQMLAFNPYGGRMSEISEFAKPFPHRSGNIAKIQYEVNWD  
 At26 1 SIWKKMIELEIPTMAFNPYGGEMGRISSTVTPFPYRAGNLWKIQYGANWR  
 At27 1 GIFKRLIEGNTTFLNWTYPYGGMMSKIPESALPFPHRNGTLFKILVYANWL  
 EcBBE 1 GLLERLSKEPNGFIALNGFGGQMSKISSDFTPFPHRSGTRLNVEYIVAWN  
 PsBBE 1 HALEMELS EQPGGFIALNGFGGK MSEISTDFTPFPHRKGTCLMFEYIIAWN

MS59 51 DLSDEAENRYLNFTRLMYDYMTPFVSKNPRKAFLNYRDLDIG - INSHG - -  
 WL64 51 ELGVEAANRYLNFTRVMYDYMTPFVSKNPREAFLNYRDLDIG - VNSHG - -  
 At26 51 DET - - LTRYMELTRKLYQFMTPFVSKNPRQSFNRYRDVDLG - INSHNG -  
 At27 51 END-KTSSRKINWIKIYNMAYPVSSNPRQAYVNYRDLDFG - QNKNN - -  
 EcBBE 51 QSEQKKKTEFLDWLEKVEYFMKPFVSKNPRLG YVNHIDLDLGGIDWGNKT  
 PsBBE 51 QDEESKIGEFSEWLAKFYDYLEPFVSKPRVGYVNHIDLDLGGIDWRNKS

MS59 98 -RNAYTEGMV-YGHKYFKETNYKRLVS VKTKVDPDNFFRNEQSIP TLSS -  
 WL64 98 -KNAYGEGMV-YGHKYFKETNYKRLTMVKTRVDPSNFFRNEQSIP TLSSS  
 At26 97 KISSYVEGKR-YGK KYFAG-NFERLVKIKTRVDSGNFFRNEQSIPVLP - -  
 At27 97 AKVNFIEAKI-WGPKYFKG-NFDRLVKIKTKVDPENFFRNEQSIPPMFY -  
 EcBBE 101 VVNNIAEISRSWGESYFLS-NYERLIRAKTLIDPNNVFNHPQSIPPMANF  
 PsBBE 101 STTNAVEIARNWGERYFSS-NYERLVKAKTLIDPNNVFNHPQSIPPMMKF

MS59 -----  
 WL64 146 WK-----  
 At26 -----  
 At27 -----  
 EcBBE 150 D - - YLEKTLGSDGGEVV I  
 PsBBE 150 EEIYMLKEL-----

Fig. 10-2

EXPRESS MAIL LABEL  
NO.: EJ405329117US